SCIENTIFIC AGRICULTURE

Vol. 22 JULY, 1942 No. 11

HYDROGEN-ION CONCENTRATION AND WEED CONTROL[1]

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[Received for publication February 13, 1942]

Following the introduction of the Hydrogen-ion hypothesis into the field of plant science, information was published (9) suggesting that soil acidity had a definite bearing on weed control. In the ensuing years this so-called acid theory gained considerable prominence in connection with lawns and golf greens but some investigators have claimed it a fallacy. A literature survey has failed to reveal any attempt to determine the respective tolerances of lawn weeds and grasses to Hydrogen-ion concentration as an isolated variable in nutrient cultures. Since many lawn weeds are very difficult to control it was deemed advisable to obtain more definite information on this question and so the following investigations were undertaken. The plants used for experimentation were brown top or colonial bent (*Agrostis tenuis* Sibth.), Kentucky blue grass (*Poa pratensis* L.), common dandelion (*Taraxacum palustre* (Lyons) Lam. & DC. var. *vulgare* (Lam.) Fern. (*T. officinale* Weber)), and white clover (*Trifolium repens* L.).

REVIEW OF LITERATURE

Hydrogen-ion Concentration and Weed Control

Hartwell and Damon (9) found that by using an acid-reacting fertilizer, ammonium sulphate, a degree of acidity developed which checked dandelions, clover, and Kentucky blue grass but was not especially detrimental to the growth of bent and red fescue. Garner and Damon (7) gave the results obtained by dressing various lawn grasses from 1905 to 1928 with fertilizer mixtures calculated to affect the soil reaction. They found that bent grasses were particularly tolerant to acid soil reactions, that Kentucky blue grass could not persist in acid soils as well as the acclimated bent grasses, and that the higher the acidity the fewer the weeds persisting, although no species was entirely eliminated in the range pH 4.2 to 8.0. Mann (14) studied the weed herbage in a field which had been under continuous wheat and barley cropping for over 50 years with various manurial treatments. Soil acidity brought about by the addition of ammonium sulphate decreased all the weeds except *Spergula arvensis*. The effect of lime on the weed flora was almost entirely governed by the change induced in the acidity. Gilbert (8) from an analysis of 22 Rhode Island golf courses found a relationship between the soil acidity from the use of sulphate of ammonia and the discouragement of weeds. Clouston (4) stated that an acid soil is advantageous in the production of a turf since grasses such as

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A portion of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.
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Agrostis and *Festuca* grow well naturally, under acid conditions and can withstand a degree of acidity in the soil which is harmful to many of the broad-leaved weeds such as plantains and clovers.

Oakley (18) found that on newly established plots the acid-reacting fertilizers gave a turf of good quality and prevented the invasion of weeds while the alkaline-reacting fertilizers allowed weeds to become established. Dawson and Greig (6) also found that an acid condition of the surface soil inhibited the invasion of weeds.

Robertson and Stewart (19) however, claimed that applications of ammonium sulphate, sulphuric acid, and flowers of sulphur all increased the acidity of the soil, but the effects on the weed flora varied, some decreasing others increasing. They concluded therefore that weed eradication was not brought about by soil acidity. Dawson and Greig (6) observed that applications of ammonium sulphate to weedy turfs decreased the weeds but the checking was not due to induced acidity as the weeds were still destroyed when the area was heavily limed prior to treatment with the fertilizer. Monteith (15) discussed at some length the acid theory of weed control in bent turf by the continued application of sulphate of ammonia and warns that such a treatment usually leads to injury of even bent grasses. He mentions that on some putting greens the application of sulphate of ammonia improved the turf and reduced the weed content although the soils did not become more acid.

Welton (31), Sprague and Evaul (24), and Sprague (23) claimed that weeds as a group are just as tolerant of acidity as are lawn grasses and they therefore question the efficiency of soil reaction in controlling all weeds. Sprague (23) also maintained that the acid soil theory of weed control is erroneous and that although bent grasses and fescues are more tolerant of acidity than Kentucky blue grass or weeds, even the acid tolerant grasses produce a sturdier growth on mildly acid or neutral soils which is better able not only to compete with weeds but also to withstand unfavourable climatic conditions and disease.

Hydrogen-ion Concentration and Growth of Roots

There are many references in the literature giving so-called optimum reaction ranges for the growth of plants but with the vast majority of these dry weight or crop yield was the criterion used to determine the optimum range, and little or no attention was paid to the effect on root growth. The limits of growth with respect to concentration of Hydrogen-ions in nutrient solutions found by various investigators are given in the following table:

Author	Reference	Plants used	Limit of growth (pH)
Howell	13	Western yellow pine	2.7
Salter and McIlvaine	20	Wheat, soy beans, corn and alfalfa	2.96
Strugger	25	Sunflower	3.0
Tarr and Noble	26	Wheat, soy beans and corn	3.0
Hoagland and Arnon	12	Tomato, lettuce, rhubarb and Bermuda grass	3.0
Wilson	32	Onion	3.5

Coggeshall (5) noted that to give 100% retardation of growth of white lupine seedlings required the following pHs for the various acids: propionic 3.3, sulphuric 3.35, acetic 3.6, and butyric 3.74.

Many authors including Bates (2), Coggeshall (5), Hoagland (11), Howell (13), Müller (16), Singh and Mitra (22), Watenpaugh (30), and Wilson (32) have stated that extreme acidity greatly reduces the extent of the plant root system, but with the majority of these this finding was incidental to the problem at which they were working. According to Hoagland and Arnon (12) and Truog (27), decidedly adverse effects from the Hydrogen-ion concentration only occur at extreme acidities, and within a considerable range fluctuations of the pH of the growth medium are not detrimental to the growth of plants.

MATERIALS AND METHODS

In connection with these investigations culture experiments were carried out as follows. Plants of common dandelion, Kentucky glue grass, and brown top were grown from March 23 to May 4, 1937, in solution cultures at pH 3.0, 3.5, 4.0, and 5.0; from January 25 to March 4, 1938, these three species were grown in sand cultures at pH 3.0, 3.3, 3.6, and 3.9; and from March 29 to May 1, 1938, plants of Kentucky blue grass and white clover were grown in sand cultures at pH 3.5, 4.0, 4.7, and 5.4.

The experimental set-up for the water cultures was similar to that employed by Shive and Stahl (21) which provided for the plants being grown in pint sealers and permitted continuous renewal of solutions with a certain amount of aeration. The experiment was carried out in duplicate under conditions of normal daylight. In the 2 sand culture experiments which were quite similar except for the levels of Hydrogen-ion concentration, the plants were grown in triplicate in ½-gallon glazed crocks filled with acid-washed sand. The period of daylight was lengthened by the use of 250-Watt incandescent lamps from dusk until midnight.

The nutrient solutions used were modified from those of Salter and McIlvaine (20). In an attempt to secure a more stable solution, potassium acid phthalate was added as a buffer and the necessary changes of the other ingredients were made to maintain the concentration of the important elements at approximately the same level as in the original solution. In a further effort to stabilize the reaction of the solution in the sand culture experiments some of the nitrogen was supplied as NH_4. The desired Hydrogen-ion concentrations were obtained by adding a given amount

Table 1.—Composition of nutrient solutions

Chemical	Water cultures	Sand cultures
K_2SO_4	.0016 M	—
KNO_3	.0100 M	.0040 M
$(NH_4)_2SO_4$	—	.0030 M
$KHC_8H_4O_4$	.0050 M	.0050 M
K_2HPO_4	—	.0048 M
$MgSO_4$	.0045 M	.0038 M
$CaCl_2$	.0025 M	.0025 M
H_3PO_4	.0087 M	.0115 M

of phosphoric acid to each solution and then finally adjusting by the addition of the required amounts of sodium hydroxide. Thus the only variant in the solutions for each experiment was sodium hydroxide. Iron was supplied as either ferric phosphate or ferrous sulphate. Tap water was used throughout for the culture solutions. The main constituents of the solutions are given in Table 1.

The plants used were all grown from seed. They varied from 10 to 15 weeks of age when placed in the cultures. The sources of the seed were as follows: that of dandelion was collected at Ottawa; the Kentucky blue grass and brown top seed was secured from the Forage Crops Division, Central Experimental Farm, Ottawa; while that of white clover was derived from two different regions, the pastures of Quebec, Canada, and of Kent, England.

With the water cultures the continuous renewal of nutrient was provided by the passage of 1000 cc. of fresh solution through the cultures every 48 hours. The method of renewal for the sand cultures was to add 500 cc. of fresh culture solution to the top of the crock each day. This displaced approximately one-half of the solution already present. To investigate the maintenance of solution Hydrogen-ion concentration levels frequent determinations were made with a quinhydrone potentiometer on the run off solutions. The acidity of the solutions decreased in passing through the cultures but with the most acid solutions employed the change was rarely greater than 0.05 pH while with those at pH 5 changes of 0.1 and even of 0.2 pH were not uncommon.

The Hydrogen-ion concentration of the solutions therefore did not change a great deal in passing through the cultures. Arnon (1) however, has pointed out that this is no indication that the pH in the vicinity of the root is the same as that of the solutions applied. Nightingale (17) found in connection with the nutrition of apple trees that "although the nutrient solutions after passing through the sand did not vary more than 0.1 pH from that initially supplied, the absorbing surfaces of the fine roots of the sand cultures supplied ammonium sulphate at pH 6.0 were relatively acid (pH 4.2). Those of the calcium nitrate series at pH 4.5 were relatively less acid (pH 5.6)". Thus the roots may have actually been growing in Hydrogen-ion concentrations quite different from those of the solutions added to the cultures and therefore the conclusions arrived at when comparing different species of plants might prove incorrect. To investigate whether this factor was complicating the issue an experiment was carried out comparing the tolerance of excised roots of the various plants to acidity. Dandelion, Kentucky blue grass, and brown top plants were grown from May 13 to July 31, 1938, under uniform conditions in pint sealers. Shives' R_5C_2 solution was used as nutrient. The roots were then cut into sections approximately two inches in length and placed in varying normalities of hydrochloric and acetic acids. At specified intervals sections were removed from the acids and tests carried out to determine if their cells were dead or alive.

In order to study the effect of the Hydrogen-ion concentration on germination and the ability of seedlings to establish themselves an experiment was carried out from April 10 to May 6, 1938, simultaneously with the second sand culture experiment. The same nutrient solutions were used

at pH 3.5, 4.0, 4.5, and 5.0. Fifty seeds of dandelion, Kentucky blue grass, and brown top were placed on acid washed sand in 3-inch waxed pots. To maintain the reactions of the cultures at a fairly constant level 30 cc. of solution was added to the surface of the sand once or twice daily and allowed to percolate.

RESULTS

The Effect of Hydrogen-ion Concentration on Growth

Comparisons of the average growth per plant produced by dandelion, Kentucky blue grass, and brown top during the solution and sand culture experiments are given in Tables 2 and 3 respectively. Similar data for Kentucky blue grass, Quebec white clover, and Kentish white clover grown in sand cultures are given in Table 4.

TABLE 2.—COMPARISON OF GROWTH PRODUCED BY PLANTS OF DANDELION, KENTUCKY BLUE GRASS AND BROWN TOP DURING SIX WEEKS IN WATER CULTURES AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Solution pH	Number of leaves			Length of longest new root in cm. *			Dry weight in gm.					
							Dandelion		Blue grass		Brown top	
	Dandelion	Blue grass	Brown top	Dandelion	Blue grass	Brown top	Roots	Tops	Roots	Tops	Roots	Tops
3.0	0	42	76	0.0	1.0	1.0	0.02	0.02	0.02	0.24	0.08	0.33
3.5	4	61	89	1.0	8.0	15.0	0.10	0.05	0.12	0.63	0.17	0.61
4.0	15	85	120	8.0	23.0	26.0	0.69	0.60	0.24	1.13	0.35	1.43
5.0	13	94	154	10.0	18.0	16.0	0.50	0.54	0.30	1.28	0.42	1.39

* Refers to adventitious roots of grasses and secondary roots of dandelion.

TABLE 3.—COMPARISON OF GROWTH PRODUCED BY PLANTS OF DANDELION, KENTUCKY BLUE GRASS AND BROWN TOP DURING FIVE WEEKS IN SAND CULTURES AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Solution pH	Number of leaves			Length of longest new root* in cm.			Dry weight in gm.					
							Dandelion		Blue grass		Brown top	
	Dandelion	Blue grass	Brown top	Dandelion	Blue grass	Brown top	Roots	Tops	Roots	Tops	Roots	Tops
3.0	0	8	5	0.0	0.5	0.5	0.02	0.01	0.02	0.04	0.01	0.02
3.3	1	12	22	0.3	1.2	1.5	0.04	0.03	0.04	0.17	0.04	1.19
3.6	1	21	26	2.5	5.1	3.7	0.17	0.07	0.09	0.24	0.05	0.25
3.9	2	20	33	6.0	7.9	7.0	0.30	0.10	0.14	0.34	0.08	0.30

* Refers to adventitious roots of grasses and secondary roots of dandelion.

TABLE 4.—COMPARISON OF GROWTH PRODUCED BY PLANTS OF KENTUCKY BLUE GRASS, QUEBEC WHITE CLOVER AND KENTISH WHITE CLOVER DURING FIVE WEEKS IN SAND CULTURES AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Solution pH	Number of leaves			Length of longest new root in cm.			Total dry weight in gm.		
	Blue grass	Quebec clover	Kentish clover	Blue grass	Quebec clover	Kentish clover	Blue grass	Quebec clover	Kentish clover
3.5	29	0	0	5.5	0.0	0.0	0.46	0.01	0.00
4.0	40	8	4	8.4	2.8	2.8	0.59	0.20	0.13
4.7	44	48	26	9.4	7.3	7.0	0.86	1.11	0.54
5.4	35	36	25	9.8	6.8	6.6	0.87	0.80	0.40

From these results it is apparent that the roots of the majority of the plants increased at least a slight amount in weight at pH 3.0. At this reaction however the dandelions did not put forth any new roots, and by the conclusion of the tests those that were present when the plants were transplanted at the beginning were dead. Even pH 3.5 was too acid for root production by white clover. With brown top and Kentucky blue grass the roots that were present at the start of the experiment were still turgid and firm at the conclusion, but they were unable to take up the vital stain neutral red and so were apparently dead. During the experiment these grasses produced new short, stub-like adventitious roots which in the sand cultures extended 0.5 cm. into the sand but in the solution cultures only grew to the surface of the solution. No doubt evaporation from the surface of the sand along with the algae that were present produced conditions allowing their development in the sand cultures. Thus while the grasses were able to exist at this pH during the experiments they were not able to produce roots to any depth into either the solution or sand cultures. It would therefore appear as if a pH of 3.0 was toxic for the roots of common dandelion, white clover, Kentucky blue grass, and brown top.

At pH 4.0 however all 4 plants were able to grow a fair amount and produce new roots well into the culture medium. Thus it would appear as if the toxic point for roots of these plants would occur between pH 3 and pH 4 under the conditions of these experiments. This is in agreement with the limits of growth found by other authors and given in the review of the literature.

It was quite evident that the 2 grasses were better able to withstand extreme acidity than was dandelion or white clover. At pH 3.5 the white clover roots died. Also at pH 3.5 in water cultures and pH 3.6 in sand cultures a few of the dandelions died and the remainder did not produce typical root systems. Their tap roots thickened up considerably, accounting for three-quarters of the total increase in dry weight, but they tapered quickly at the distal end and there were very few secondary roots arising from them. These secondary roots as well as being short and stout had no smaller branch roots or root hairs present. In comparison at these reactions the grasses produced a large number of new, fairly long adventitious roots with quite a large number of very short, hair-like laterals. In addition the brown top roots had a large number of root hairs present in the sand cultures. Even at approximately pH 4.0 in the sand cultures the secondary roots of the dandelion and the adventitious roots of white clover were comparatively few in number, quite stout with no fine branches or root hairs, while the grasses were able to produce a large number of long finely branched roots with root hairs. However from the results obtained it would appear that the death point would not vary more than one-half of a pH with the toxic point for dandelion and white clover nearer pH 4 than 3 and for the grasses nearer pH 3 than 4.

No great difference could be observed between the ability of Kentucky blue grass and brown top to tolerate acidity. Brown top was found to produce fine branch roots and root hairs at a somewhat lower pH than Kentucky blue grass which would no doubt give it a slight advantage in the extreme acid mediums. In some instances however Kentucky blue

grass was superior to brown top with regard to amount of dry weight produced. Dandelion and white clover were not grown in the same experiment so it is difficult to compare their ability to withstand acidity. From a comparison of the results from the different experiments however dandelion would appear to have a slight advantage over white clover in this respect.

It was also evident from the results obtained that Hydrogen-ion concentrations below the critical point had an inhibiting effect on root development. From Tables 2, 3, and 4, it will be found for instance that as the acidity of the cultures decreased the roots of all plants increased in length and dry weight. In the second sand culture experiment particular attention was paid to the effect of the Hydrogen-ion concentration on the morphology of the root system of Kentucky blue grass. At pH 3.5 a fair number of roots were produced during the experiment but they were short, fairly stout and brownish white in colour. Some of these roots possessed very short laterals and a few root hairs, but the small branch roots ordinarily present in a root system were almost entirely absent at this Hydrogen-ion concentration. At pH 4.0 the roots produced were more numerous, and longer and whiter in colour although a few were still tinged a brownish white. The laterals present on these roots were twice as numerous and double the length of those produced at pH 3.5. Root hairs were quite plentiful too. At pH 4.7 the roots produced during the experiment were not a great deal longer than those grown at pH 4.0 but were much more numerous, white in colour and with many more laterals present. Root hairs were plentiful and a few of the lateral roots were branched again. The roots produced at pH 5.4 were quite similar to those grown at pH 4.7. Thus as the growth medium became less acid the roots were more numerous longer and possessed a greater number of fine laterals. These lateral roots in turn became more numerous, longer, and developed smaller branch roots.

In this experimental work the major emphasis was to determine the critical Hydrogen-ion concentration for the various plants so little attention was paid to the question of where on the pH scale inhibition of root development first occurred. From general observations made however it is doubtful if the growth of plant root systems are inhibited to any great degree in nutrient cultures until the Hydrogen-ion concentration is greater than 10^{-5}. This confirms the conclusions reached by Hoagland and Arnon (12) and Truog (27) that Hydrogen-ions only exert a direct toxic action at extreme acidity. It was also fairly evident that the inhibition of root development of dandelion and white clover would start at a higher pH than for brown top or Kentucky blue grass.

Tolerance of Excised Roots to Acids

The results obtained when investigating the tolerance of excised roots of dandelion, Kentucky blue grass, and brown top to hydrochloric and acetic acids are given in Table 5.

These results corroborate those obtained when the 3 plants were grown in solution and sand cultures at different Hydrogen-ion concentrations in that the roots of the 2 grasses Kentucky blue grass and brown top were better able to endure hydrochloric and acetic acids for a given length of time than were those of common dandelion. For instance N/1000 HCl killed the dandelion roots almost immediately, whereas those of the grasses

TABLE 5.—CONDITION OF ZONE OF ELONGATION OF DANDELION, KENTUCKY BLUE GRASS, AND BROWN TOP ROOTS AT INTERVALS AFTER PLACING IN ACIDS OF VARYING NORMALITIES

Acid	Normality of acid	pH of acid	Number of hours roots were exposed to acids					
			Dandelion		Kentucky blue grass		Brown top	
			12	24	12	24	12	24
HCl	N/100	2.01	Dead	Dead	Dead	Dead	Dead	Dead
	N/400	2.57	Dead	Dead	Maj. alive	Half alive	Dead	Dead
	N/800	2.86	Dead	Dead	Alive	Maj. alive	Maj. alive	Maj. dead
	N/1000	2.96	Dead	Dead	Alive	Maj. alive	Alive	Alive
	N/4000	3.20	Alive	Alive	Alive	Alive	Alive	Alive
CH_3COOH	N/100	3.30	Dead	Dead	Dead	Dead	Dead	Dead
	N/400	3.60	Dead	Dead	Half alive	Half alive	Maj. alive	Maj. dead
	N/800	3.75	Alive	Maj. dead	Alive	Half alive	Alive	Maj. alive
	N/1000	3.84	Alive	Maj. dead	Alive	Half alive	Alive	Maj. alive
	N/10000	4.20	Alive	Alive	Alive	Alive	Alive	Alive
Distilled water		—	Alive	Alive	Alive	Alive	Alive	Alive

were still alive after 24 hours exposure. A similar advantage was exhibited by the 2 grasses in N/400 and N/800 acetic acid. As to variation between the two grasses the differences with acetic acid were not very great but with hydrochloric the Kentucky blue grass appeared to have a slight advantage.

White clover was not included in the above experiment with excised roots but at a later date similar tests were carried out with this species. The zone of elongation area of the white clover roots was dead after 24 hours exposure to N/8000 HCl at pH 3.9 and N/2000 CH_3COOH at pH 4.0. It was apparent therefore that the white clover roots could not endure as high a concentration of hydrochloric or acetic acid for a given length of time as could those of dandelion which is in agreement with results obtained when these plants were grown in sand cultures at different Hydrogen-ion concentrations.

The Effect of Hydrogen-ion Concentration on Germination

The results obtained when seeds of dandelion, Kentucky blue grass, and brown top were germinated in sand watered with nutrient solutions of pH 3.5, 4.0, 4.5, and 5.0 are given in Table 6.

The inhibiting effect of the highly acid medium on germination was least for brown top and greatest for dandelion with Kentucky blue grass between the two. In an experiment not included in this treatise, dandelion seed collected at a different time from that used above germinated 85% on the surface of a nutrient solution at pH 3.0, and there was no significant difference in the germination of this dandelion seed within the range of pH 3.0–5.8. What accounted for the difference in these two experiments is not known, but it was apparent that under certain conditions a concentration of Hydrogen-ions of 10^{-3} in a nutrient solution had no appreciable effect on the germination of dandelion seed. Extreme acidity is therefore necessary before germination is affected to any degree. When one considers what is involved in the process of germination this is not at all surprising.

TABLE 6.—EFFECT OF HYDROGEN-ION CONCENTRATION ON GERMINATION AND DEVELOPMENT OF RADICLES OF DANDELION, KENTUCKY BLUE GRASS, AND BROWN TOP

Soln. pH	Percentage germination			Growth of radicle		
	Dandelion	Kentucky blue grass	Brown top	Dandelion	Kentucky blue grass	Brown top
	%	%	%			
3.5	6	36	94	Completely checked	Completely checked	Severely checked
4.0	16	80	84	Completely checked	Checked slightly otherwise normal	Checked slightly otherwise normal
4.5	52	76	92	Checked slightly otherwise normal	Normal	Normal
5.0	55	72	88	Normal	Normal	Normal

A pH of 3.5, however, had a definite inhibiting effect on the development of the radicles of dandelion, Kentucky blue grass, and brown top seedlings. The amount of inhibition was not the same for the 3 species as both grasses were able to produce primary roots with root hairs into sand watered with a nutrient solution of a higher Hydrogen-ion concentration than was dandelion. Grass seedlings can therefore establish themselves in a more acid medium than can those of common dandelion. Since seeds of all 3 plants germinated at a Hydrogen-ion concentration too acid to allow growth of their radicles, root growth is apparently more sensitive to acidity than is germination. Similar results were obtained by Bryan (3), Hixon (10), Müller (16), and Salter and McIlvaine (20).

DISCUSSION

From the results obtained during the course of these experiments it was evident that the 2 grasses Kentucky blue grass and brown top had a greater tolerance of Hydrogen-ions than either dandelion or white clover. One might conclude therefore that acidity would be beneficial in controlling these weeds in a lawn. The critical reaction for all the species however was between pH 3.0 and 4.0 which is more acid than most arable soils and very definitely more acid than the great majority of surface soils under cultivation. It is extremely doubtful therefore whether the difference between the grasses and weeds is great enough for acidity, per se, to act as a weed controlling factor. Acidification of the soil to a degree approaching the critical reaction would not only be prohibitive as to cost but in the final analysis would result in a poor physical condition of the soil and a lowering of the base saturation through a depletion of soil bases.

It should be pointed out that in these investigations the various species were grown in separate containers and not in competition with each other. It was found that the growth of roots was inhibited by concentrations of Hydrogen-ions below the critical point and that at any one concentration where inhibition occurred the normal root development of dandelion was more adversely affected than was that of the grasses. Thus if these plants were growing in competition with each other the reaction of the growth medium if of only medium acidity might play a part in their distribution.

Truog (27), de Vries, (29) and Van Dersal (28) have pointed out the importance of competition in connection with the distribution of plants. Van Dersal found that "the competition of grasses planted in mixtures may materially change the reaction at which one or the other of the species succeeds". The part that competition may play in connection with acidity and weed control requires further experimentation.

The results from the germination experiment indicate that grass seedlings are able to establish themselves at a Hydrogen-ion concentration too high for the dandelion seedlings to do so. It was also evident when these results were compared with those obtained in the other culture experiments that older plants of the 2 grasses were able to make very good growth at reactions which were too acid for young dandelion seedlings to grow. Therefore if the surface layer of soil were maintained at a high Hydrogen-ion concentration the indications are that it would prevent such weeds as dandelions from becoming established and thus acidity could be beneficial from the standpoint of preventing weed invasion. The results of Oakley (18) and Dawson and Greig (6) suggest that this is possible. The threat of weed infestation is most serious on new seeding and in the case of established lawns where the grass is in a poor condition. If therefore the surface layer of the soil were made definitely acid during the time of year when weed seeds were germinating it might be possible to prevent the weeds from establishing themselves without either preventing grass seedlings from doing so or affecting the growth of the grasses already present. If this were repeated year after year however the underlying soil would become acid leading to the depletion of soil bases etc. It should therefore be looked upon as an emergency program to be carried out until a good sward of grass is obtained rather than a regular lawn practice.

SUMMARY

Plants of common dandelion, white clover, Kentucky blue grass, and brown top were grown in sand and solution cultures at various acidity levels to determine their respective tolerances to the Hydrogen-ion concentration as a prelude to evaluating this factor in connection with control of weeds.

The toxic or critical point for the 4 species when grown separately occurred between pH 3 and pH 4. The grasses exhibited a slight advantage with regard to tolerance of acidity since their critical point was nearer pH 3 than pH 4 while that of dandelion and white clover was nearer pH 4 than pH 3.

The exposure of excised roots grown under uniform conditions to hydrochloric and acetic acids confirmed the results obtained from the cultures in that both grasses were able to withstand a higher concentration of acid for a given period of time than was either dandelion or white clover.

Seedlings of Kentucky blue grass and brown top were able to establish themselves in a medium of greater acidity than was common dandelion. Growth is more sensitive to acidity than is germination as the seeds of all species tested germinated at Hydrogen-ion concentrations too high for growth to take place.

Inasmuch as the critical point for all plants is more acid than most soils it is doubtful if the difference in tolerance between the grasses and weeds is great enough for acidity, per se, to act as a weed controlling factor. It is possible however that if the surface layer of soil were acidified it would prevent weed invasion while a good turf was being established.

ACKNOWLEDGMENT

The author is deeply indebted to Dr. G. W. Scarth, McGill University, Montreal and to Dr. H. T. Güssow and Mr. H. Groh of the Division of Botany, Department of Agriculture, Ottawa, for valuable assistance, suggestions, and encouragement during the progress of these experiments.

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CULTURAL STUDIES WITH BARLEY

II. DIFFERENTIAL RESPONSES TO FERTILIZER TREATMENT AND RATE OF SEEDING WITH RESPECT TO YIELD[1]

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[Received for publication November 10, 1941.]

In the preceding paper in this series (7) the differential yield responses of three varieties of barley to dates of seeding were discussed. As pointed out at that time two other factors were involved in the study, namely fertilizer treatment and rates of seeding. These two are dealt with in the present paper. The data and discussion presented herewith deal not only with each separate factor, but also with the differential response of each in relation to the other factors, or, in other words, with the interaction with all of the other factors involved in the entire investigation.

REVIEW OF LITERATURE

Although the amount of available information dealing with the interrelations of the particular cultural factors involved in the present investigation is limited, much work has been done on various aspects of fertilizer treatment with barley. Hopkins (4) analyzed and reported upon the results of an experiment undertaken by T. J. Harrison in Manitoba, in which the effect of nitrogenous fertilizers, phosphate, and potash applied at seeding time and at heading time were studied. The yields of the barley crop sown on summer-fallowed land were determined in the year that the fertilizers were applied, and also the yields of a second crop of barley upon the same land. The latter yields of course tested the residual effects of the fertilizers. He showed that an increase of 5½ bushels per acre was obtained from phosphate applied at seeding time in 1926 to the crop sown on summer-fallowed land. No increase was obtained from either nitrogen or potash alone or from a combination of all three fertilizers. In 1927 on the other hand, no increase was obtained from any of the three fertilizers applied alone to the crop during the year, but the combination of the three produced an increase of 7½ bushels. In the case of the second crop barley following fertilization of the preceding crop, a pronounced residual effect of nitrogen was obtained. Although Hopkins concluded from the 2-years' results that, under soil conditions similar to those involved in the experiment, even heavy top dressings will have relatively small and uncertain effects on the yield of barley on summer-fallowed land, he called attention to Ellis' work (2) as suggesting that drilling in moderate amounts below the seed, may bring about greater responses. Richardson and Fricke (8) and Richardson and Gurney (9) (Australia) found a marked response in barley yields to applications of sulphate of ammonia (superimposed on a basal

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dressing of 2 cwt. per acre of superphosphate). They also found that when amounts ranging from 141 to 600 lbs. of sulphate of ammonia were applied increases in yield took place from applications up to 425 lbs. From that point on there was a reduction. They pointed out that "increments in yield due to successive moieties of fertilizer became progressively smaller in accordance with the law of diminishing returns". Russell and Bishop (13) reviewing the results of the long continued experiments of Rothamsted, England, concluded that under English agricultural conditions nitrogen was the only one of the three fertilizer elements (N. P. and K.) that was consistently effective in increasing yields of barley. Russell (10), (11) pointed out that the average increase given by sulphate of ammonia was the same whether phosphate and potash were added or not. He also showed that increases from this fertilizer were obtained whether the barley was grown after a straw crop, after roots fed off, or after potato and beet crops. Russell (11, 12) and Russell and Watson (14, 15) called attention to the fact that the recommendations based upon the Hoos field results as reported by Lawes and Gilbert (6) were not supported by those from the later experiments. Those recommendations were that manuring of barley should be mainly phosphatic, nitrogen being given only in certain circumstances, and were based, in part, upon the observed reduction in yield of 7 cwt. per acre where phosphate was withheld from the fertilizer mixture. Contrasting with these results, those under the later experiments showed that the reduction in yield due to omission of superphosphate was small as compared with that due to the omission of nitrogenous fertilizer. Commenting on the discrepancy between the older and the more recent results the authors suggested that the reasons may be: (1) that modern varieties such as Plumage Archer can stand more nitrogen because of stiffer straw; (2) that heavy soils such as Hoos field usually respond well to phosphate and also that Hoos field was more exhausted of phosphate than is usually the case; and (3) that on account of liberal applications made to roots when these are grown in rotation with barley, the latter crop finds enough phosphate for its needs. Referring to the observation that phosphate sometimes depresses yield, they suggested that if this effect is real it may be due to the fact that, under certain conditions which bring about ripening that is already too early for optimal yield on light soils, the effect of the phosphate is to further hasten maturity thereby further curtailing yield. Russell and Watson (14) discussing the variation in response to nitrogenous fertilizers due to season and rainfall point out that the increments in yield were less in wet springs than in dry ones, and also that although the increments per unit of added nitrogen at different centres tended to be similar over a period of years, they tended to be greater in years of low basal yield and less in years of high basal yield. Burgevin and Sarazin (1), referring to the relation of time of sowing to nitrogen response found that while absorption of nitrogen by the plant was practically independent of time of sowing, the amount of dry substance produced was less the later the sowing date. They accounted for this seeming discrepancy by showing that late sown barley showed an increase in nitrogen percentage as compared with early sown barley. Russell and Watson (14) also showed that increase in yield from nitrogenous fertilizer was greatest under early sowing.

In this connection they pointed out the interesting fact that among three varieties involved in the experiment, namely Plumage Archer, July and Victor, July showed much the least response to difference in date of sowing.

It is obvious that the question of need for fertilization is a local one. Not only soil, but also climate, season, locality, date of seeding, and variety exert important influences. The needs of the crop must be determined in the light of each and all of those factors. The results obtained in any given locality may not be applied to another without verification against the background of the conditions prevailing in the new locality.

Rate of seeding data from a number of different sources are summarized by Hughes and Henson (5). Out of a total of 12 different experiments reported in which rates from 4 to 16 pecks were tried, 5 showed optimum yields for 8 pecks, 3 for 6 pecks, 2 for 4 pecks, 1 for 10 pecks and 1 for 16 pecks. In general the lower rates gave optimum results under dry land conditions and the higher ones under humid conditions. In the one instance in which 10 pecks produced the highest yield, the crop was grown under irrigation. Thayer (16) reporting upon results in Michigan were 4 varieties were involved in the experiment with six seeding rates ($\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$ and 3 bushels per acre), showed that Glabron and Michigan 2-row returned the highest yield when sown at $1\frac{1}{2}$ bushels, Spartan at 2; and Wisconsin No. 38 at 1 bushel per acre. All varieties returned the lowest yield at $\frac{1}{2}$ bushel, and all but Spartan, the next lowest, at 3 bushels. Spartan, a 2-row barley was the largest seeded variety of the four.

MATERIALS AND METHODS

The plan of the present investigation was given in detail in the preceding paper (7). It involved 3 dates of seeding, 3 varieties, 3 rates of seeding and 3 fertilizer treatments. The fact was stressed that the design of the experiment was such that each level of each factor could be studied at every level of the other factors. The three varieties were O.A.C.,21, Mensury Ottawa 60 and Gartons. Four stations were involved, namely, Winnipeg, Carman, Newdale and Swan River, exactly the same procedure having been followed at each. The study was continued over the three years 1937, 1938 and 1939. Two of these, namely 1937 and 1938, were severe rust years, and the third 1939 was relatively rust free and otherwise normal. Meteorological data, including temperature and precipitation records for each of the years were presented in tabular form in the previous paper (7).

The three fertilizer treatments really consisted of two kinds of fertilizer versus no fertilizer. The two kinds of fertilizer consisted of two combinations and rates. They were 96 lbs. per acre of 16-20-0 and 40 lbs. of 11-48-0. The two treatments provided for equal amounts of phosphate (P^2O^5), while the 96 lb. rate applied more than 3 times as much nitrogen as the 40 lb. rate. The fertilizers were applied in drills at the time of reading. The plan of the experiment was such that there were as many check plots, or plots receiving no treatment, as there were plots of each of the two kinds of treatment.

The three rates of seeding were 1 bushel, $1\frac{3}{4}$ bushels and $2\frac{1}{2}$ bushels per acre.

RESULTS AND DISCUSSION

Effect of Fertilizer Treatment

In Table 1 the yields for each of two fertilizer treatments and for the comparable checks (no fertilizer) are presented by stations as means over all other factors (rates, dates, varieties and years). The differences between the two rates are uniformly small. They are consistently in favour of the 96-pound rate of 16-20-0, but nevertheless are not significant statistically as is shown below.

Table 1.—Yields from two fertilizer treatments and from no treatment as means over all other factors (rates, dates and varieties)

	(40) 11-48-0	(96) 16-20-0	No fertilizer
	(Bu. per acre)	(Bu. per acre)	(Bu. per acre)
Winnipeg	37.9	38.7	36.2
Carman	30.2	31.9	24.2
Newdale	23.5	23.7	20.6
Swan River	49.7	50.2	38.9

In Appendix Table 11, the analyses of variance are given for all factors and interactions covered in this paper. Because of the fact that the differences between the two fertilizer treatments were found not to be significant the results for both were combined for the purposes of studying interaction between fertilizer and the various other factors involved. However, the evaluation of the difference between the two fertilizer treatments and also the difference between treatment and no treatment is shown by the following supplementary data calculated by standard statistical procedure (Table 1b).

Table 1b.—Analysis of variance for fertilizer treatments

	Winnipeg		Carman		Newdale		Swan River	
	DF	Mean Square	DF	Mean Square	DF	Mean Square	DF	Mean Square
Fert. vs. no fert.	1	667.47	1	4511.14	1	640.94	1	13215.07
96 lb.s vs. 40 lbs.	1	79.94	1	179.26	1	.42	1	25.22
Greatest pertinent interaction	4	173.05	4	403.96	4	98.68	4	1303.48
Mean of all pertinent interactions	142	62.25	94	97.77	94	20.94	94	134.56
Residual error	432	32.30	288	9.50	288	18.00	288	30.60

Three error mean squares are presented in Table 1b. The first is the largest of the mean squares for the interaction items in which fertilizer appears. Reference to Appendix Table 11 will show that there are 15 such items. The use of this as error involves the greatest bias in the direction of non significance of the item with which it is compared. The second is the average of all the fifteen pertinent interaction mean squares (the total sums of squares divided by the total degrees of freedom involved), and the third the residual error. The latter involves all the degrees of

freedom that remain after all the treatment and interaction mean squares have been accounted for and represents the least severe test of significance of all the errors that might be selected for the purpose.

It is evident at a glance that the difference between the two rates of fertilizer is not significant at any of the stations when tested by either the first or second of the above described errors.

We may now compare the results for fertilizer versus no fertilizer. The data in Table 2 afford such a comparison. These are the station means appearing at the bottom of the respective "fertilizer" and "no fertilizer" columns at the extreme right of Appendix Table 12. They show that the increased yields due to fertilizer treatment varied from 2 bushels per acre at Winnipeg to 11 bushels at Swan River. In relation to the total yields in each case the increases were large at Swan River and Carman, considerable at Newdale and small at Winnipeg.

TABLE 2.—FERTILIZER VS. NO FERTILIZER. YIELDS AS MEANS OVER ALL OTHER FACTORS (RATES, DATES AND VARIETIES)

	Fertilizer	No fertilizer
	(Bu. per acre)	(Bu. per acre)
Winnipeg	38.2	36.2
Carman	31.0	24.1
Newdale	23.6	20.6
Swan River	49.9	38.9

The Swan River data represent only two years, the crops having failed on account of hail. At Carman the crop from the third seeding date was a total failure in 1939, because of heat and drouth, and the Carman data for that year are therefore omitted.

Referring again to Table 1b for the purpose of evaluating the significance of the differences in favour of fertilizer treatment, it is at once apparent that the differences are highly significant. The mean squares for fertilizer vs. no fertilizer are large even in relation to those for the greatest pertinent interaction m.s., and are significant to the 1% level when compared with the mean squares for the average of the pertinent interaction. There can be no doubt that the increases recorded for fertilizer treatment were real at all stations.

Differential Response of Varieties to Fertilizer Treatment

In Table 3 the yields for each of the three varieties under fertilizer and no fertilizer treatment are shown by stations, these data consisting of the station-variety means appearing in the fertilizer and no fertilizer columns at the extreme right of Appendix Table 12. The data indicate the responses of Gartons to fertilizer treatment was distinctly less marked than that of the other two varieties. This was especially true of the three stations Winnipeg, Newdale and Swan River.

TABLE 3.—RESPONSE OF VARIETIES TO FERTILIZER TREATMENT INDICATED BY MEAN YIELDS OVER THE OTHER FACTORS (RATES AND DATES)

	O.A.C. 21			Gartons			Mensury		
	Bu. per acre			Bu. per acre			Bu. per acre		
	Fert.	No fert.	Dif.	Fert.	No fert.	Diff.	Fert.	No fert.	Diff.
Winnipeg	38.4	35.0	3.4	39.1	39.8	−0.7	37.2	33.9	3.3
Carman	29.7	21.4	8.3	36.8	31.8	5.0	26.6	19.2	7.4
Newdale	23.4	20.1	3.3	24.8	23.4	1.4	22.6	18.5	4.1
Swan River	49.2	36.5	12.7	52.9	47.2	5.7	47.7	33.0	14.7
Mean									

The analyses of variance (Appendix Table 11) show that the V × F mean square is significant at the three stations Winnipeg, Newdale and Swan River, when an average interaction mean square derived in the manner explained in the preceding section, is used as error. Seven items contribute to that average. The confidence that may be placed in that evaluation of significance is emphasized by the fact that not less than five of the interaction mean squares contributing to the average at any of the three stations, showed the V × F interaction to be significant when each was used separately as error. It was not significant at Carman. It is clear that the interaction between varieties and fertilizers or more specifically the lower increase due to fertilizer obtained for Gartons as compared with the other two varieties was significant at the three indicated stations.

Interaction between Fertilizer Treatment and Date of Seeding

In view of the striking differences in yield under different dates of seeding, pointed out in the previous paper, a comparison of fertilizer treatment with no fertilizer upon the crops sown at the different dates is of special importance. For the purpose of such comparison the appropriate data are presented in Table 4. These have been taken from Appendix Table 13 where they appear as means by dates for fertilizer and no fertilizer treatment, respectively, in the last two columns. They are presented in Table 4 together with the differences between respective comparable pairs of means.

TABLE 4.—DIFFERENTIAL EFFECT OF FERTILIZER TREATMENT WHEN APPLIED TO BARLEY SOWN AT SUCCESSIVE INTERVALS OF TWO WEEKS (MEANS OVER ALL VARIETIES AND SEEDING RATES)

	Date 1 (early May)			Date 2			Date 3		
	Bu. per acre			Bu. per acre			Bu. per acre		
	Fert.	No fert.	Diff.	Fert.	No fert.	Diff.	Fert.	No fert.	Diff.
Winnipeg	46.4	42.8	3.6	39.2	38.1	1.1	29.2	27.8	1.4
Carman	45.1	33.9	11.2	30.8	26.7	4.1	17.2	11.9	5.3
Newdale	26.6	22.5	4.1	23.7	21.8	1.9	20.5	17.7	2.8
Swan River	47.8	35.8	12.0	54.6	37.0	17.6	47.4	43.9	3.5

The largest absolute increases due to fertilizer treatment took place at the first date of seeding at all stations except Swan River. At Carman the relative increase was greater at the third than at the first date. The first was by far the most favourable seeding date at these stations as

determined by mean yields over all other factors. At Swan River the greatest response took place at the second date. This was the most favourable seeding date at that station as judged by the mean yield over all other factors. It will be noted that the mean over the no fertilizer treatment shows the third to have been the most favourable seeding date. In general then the indications are that the most favourable response to fertilizer treatment took place at the most favourable seeding date.

The analyses of variance show that there was a significant differential response at Swan River and Carman. The F × D interaction is shown to be on the threshold of significance even when the largest interaction mean square is used as error. Measured by each of the other six pertinent interaction mean squares a high degree of significance is indicated. Similarly at Carmen, significance is indicated when the comparison is made with each of five of the seven pertinent mean squares. The interaction was apparently not significant at Winnipeg and Newdale.

Triple Interaction between Varieties, Fertilizer Treatment and Date of Seeding

The differences between fertilizer and no fertilizer treatment are shown by dates and varieties as well as by stations in Table 5. They are expressed in bushels and per cent. and are derived from Appendix Table 14 where actual yields are presented. They show no particular trends except the simple interactions already pointed out. At Swan River the conspicuously greater response to fertilizer treatment at the second date of seeding was maintained by all varieties. Similarly the lower response of Gartons to fertilizer treatment as compared with the other varieties was maintained at all dates. In other words there is no indication that the fertilizer × date interaction differentiated by varieties, nor that the variety × fertilizer interaction differentiated by dates.

TABLE 5.—EFFECT OF FERTILIZER TREATMENT BY VARIETIES AND DATES OF SEEDING EXPRESSED AS ABSOLUTE AND RELATIVE DIFFERENCES BETWEEN FERTILIZER AND NO FERTILIZER TREATMENT

Variety and station	Differences between fertilizer and no fertilizer treatments					
	Date 1		Date 2		Date 3	
	bu.	%	bu.	%	bu.	%[1]
Winnipeg						
O.A.C. 21	6.6	15.2	1.9	4.9	1.8	7.9
Gartons	0.9	2.2	−2.0	−5.0	−0.9	−2.3
Mensury	3.3	7.4	3.3	9.4	3.3	15.2
Carman						
O.A.C. 21	14.2	45.8	4.5	18.1	6.1	72.8
Gartons	6.7	16.2	2.8	8.3	5.3	26.0
Mensury	12.7	43.5	5.1	24.0	4.5	64.3
Newdale						
O.A.C. 21	4.8	21.3	2.1	10.0	2.9	17.4
Gartons	2.3	10.0	0.7	2.8	1.1	5.0
Mensury	5.0	22.6	3.0	15.8	4.5	31.7
Swan River						
O.A.C. 21	13.4	38.6	19.3	54.4	5.3	13.5
Gartons	5.5	13.5	12.0	27.5	−0.3	−0.5
Mensury	17.1	53.3	21.4	67.0	5.6	16.1

[1] Yields for no fertilizer = 100.

The above conclusion is supported by the fact that the analyses of variance (Appendix Table 11) show no significance for the triple interaction (V × F × D) at any of the stations even when the least severe test is made, namely when the residual error is used as the criterion.

Effect of Rate of Seeding

Summarized data covering rates of seeding are presented by stations in Table 6. They indicate a substantial increase in yield as a result of increasing the rate from 1 bushel to $1\frac{3}{4}$ bushels per acre, and only a small further increase for the $2\frac{1}{2}$ over the $1\frac{3}{4}$ bushel rate. In general the increased return from the highest over the medium rate was only a little greater than the additional amount of seed sown. As an average of all stations the net increase for the $2\frac{1}{2}$ over the $1\frac{3}{4}$ rate was $\frac{3}{4}$ bushel. The smallest net increase by stations was less than $\frac{1}{2}$ bushel (Newdale), and the largest less than $1\frac{1}{4}$ bushels (Carman).

Table 6.—Yields under different rates of seeding as means over all other factors (varieties, dates and fertilizers)

	1 bushel (per acre)	$1\frac{3}{4}$ bushels (per acre)	$2\frac{1}{2}$ bushels (per acre)
Winnipeg	34.8	38.3	39.6
Carman	26.2	29.1	31.0
Newdale	20.8	22.9	24.1
Swan River	42.8	47.3	48.7

Differential Response of Varieties to Rates of Seeding

The data in Table 7 disclose the interesting fact that Gartons stood out as showing less response to increased seeding rate than the other two varieties. The behaviour was consistent at all stations. Although, as already pointed out, there was little increase for the third over the second rate of seeding, these data show a consistently greater difference between these two rates for both O.A.C. 21 and Mensury than for Gartons.

Table 7.—Yield for each rate of seeding by varieties and stations as means over all other factors

Variety and station	1 bushel (per acre)	$1\frac{3}{4}$ bushels (per acre)	$2\frac{1}{2}$ bushels (per acre)
Winnipeg			
O.A.C. 21	33.6	38.0	40.1
Gartons	39.3	39.1	39.7
Mensury	31.5	37.7	39.1
Carman			
O.A.C. 21	23.3	27.9	29.6
Gartons	34.4	35.5	35.6
Mensury	20.8	23.8	27.9
Newdale			
O.A.C. 21	20.6	22.0	24.3
Gartons	23.7	24.5	24.8
Mensury	18.3	22.3	23.1
Swan River			
O.A.C. 21	39.4	46.7	48.6
Gartons	49.0	52.6	51.5
Mensury	39.8	42.6	46.2

The analyses of variance support the above indicated evidence of differential response at Winnipeg and Newdale where the V × R interaction is show to be significant. At the other two stations significance is not shown.

The differential behaviour does not appear to have been due to difference in size or viability of seed as between varieties.

Interaction between Date and Rate of Seeding

Since there was such a marked response to date of seeding (7) it is important to determine whether the response to different rates of seeding was greater or less at the more favourable than at the less favourable seeding dates. The information is furnished by the data in Table 8. At Winnipeg the positive response to the increased seeding rates was distinctly greater at the second and third or less favourable seeding dates than at the first. Similarly, there was a consistent relation between rate of seeding, and decrease in yield due to delayed seeding. The difference between the first and second dates and also between the first and third was much less under the higher than under the lower seeding rates. At the other three stations there appeared to be no similar trends.

TABLE 8.—YIELDS FOR EACH RATE OF SEEDING BY DATES AND STATIONS AS MEANS OVER ALL OTHER FACTORS

Date and station	1 bushel (per acre)	$1\frac{3}{4}$ bushels (per acre)	$2\frac{1}{2}$ bushels (per acre)
Winnipeg			
Date 1	44.6	45.4	45.6
Date 2	34.7	40.0	41.7
Date 3	25.1	29.5	31.5
Carman			
Date 1	37.4	41.8	44.9
Date 2	27.5	29.8	31.0
Date 3	13.6	15.5	17.2
Newdale			
Date 1	23.3	25.4	27.0
Date 2	21.0	23.5	24.7
Date 3	18.2	19.9	20.6
Swan River			
Date 1	40.8	45.2	45.3
Date 2	44.2	50.5	51.5
Date 3	43.1	46.1	49.4

The analyses of variance show that at Winnipeg the mean square for R × D is significant when tested by each of 5 out of 7 interaction mean squares involving both R and D, and by the error mean square, and confirms the above indicated conclusion that there was a distinct interaction between rate of seeding and date of seeding at that station. For each of the other three stations the analyses indicate that the interaction was not significant.

Interaction between Rate of Seeding and Fertilizer Treatment

We may next inquire whether the same seeding rate was optimum where fertilizer was and was not applied. Table 9 provides data bearing

on this point and shows that the same rate was optimum. The direction of increase was the same for the fertilizer and no fertilizer treatments at each station. There were differences in degree but these were not consistent.

The analyses of variance show that the interaction mean square for R × F is not significant.

TABLE 9.—COMPARATIVE YIELD RESPONSES TO RATE OF SEEDING UNDER FERTILIZATION AND UNDER NO FERTILIZATION (MEANS OVER ALL VARIETIES)

	1 bushel (per acre)		$1\frac{3}{4}$ bushels (per acre)		$2\frac{1}{2}$ bushels (per acre)	
	Fert.	No. fert.	Fert.	No. fert.	Fert.	No. fert.
Winnipeg	35.8	32.8	39.3	36.5	39.7	39.4
Carman	28.6	21.2	31.3	24.6	33.2	28.7
Newdale	21.5	19.5	24.0	20.8	25.3	21.6
Swan River	46.7	34.9	50.5	40.8	52.6	41.0

Triple Interaction between Rate of Seeding, Fertilizer Treatment and Variety

The conclusion given above assumes no differential response of variety since it is based upon means over all varieties. That there was such a differential response was shown in an earlier section. It was established that Gartons showed distinctly less response to increased rate of seeding than the other two varieties. The data in Appendix Table 12 make it possible to determine whether or not the statement that the seeding rate which was optimum where fertilizer was applied was also optimum where no fertilizer was applied, was equally true for all varieties.

For convenience of analysis Table 10 has been prepared from the data in Appendix Table 12. In it the optimum seeding dates have been classified under "fertilizer" and "no fertilizer" for each variety by stations. This array shows at once that, for practical purposes, the only exceptions to the statement that the same rates were optimum for both fertilizer and no fertilizer treatments were furnished by Gartons. For both of the other varieties the $2\frac{1}{2}$ bushel rate was optimum in every case except O.A.C. 21 no fertilizer at Carman, and here the difference in yield (as reference to Appendix Table 12 will show) between the $1\frac{3}{4}$ and the $2\frac{1}{2}$ bu. rate was 0.1 bushel. With Gartons, on the other hand, the optimum rate for "fertilizer" and "no fertilizer" differed in three cases, namely Winnipeg, Carman, and Newdale.

TABLE 10.—CLASSIFICATION OF OPTIMUM SEEDING RATES BY VARIETIES UNDER EACH OF FERTILIZED AND UNFERTILIZED TREATMENTS

	O.A.C. 21		Gartons		Mensury	
	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.
	bu.	bu.	bu.	bu.	bu.	bu.
Winnipeg	$2\frac{1}{2}$	$2\frac{1}{2}$	1	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$
Carman	$2\frac{1}{2}$	$1\frac{3}{4}$	$2\frac{1}{2}$	$1\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{2}$
Newdale	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$1\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{2}$
Swan River	$2\frac{1}{2}$	$2\frac{1}{2}$	$1\frac{3}{4}$	$1\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{2}$

In evaluating these apparent exceptions it should be borne in mind that substantial differences in yield were found only when the lower rate was compared with either of the higher ones. In other words, when the $1\frac{3}{4}$ bushel rate was compared with the next higher one only a small to insignificant further increase in yield took place. Therefore, if the optimum rate for each of "fertilizer" and "no fertilizer" was indicated as either $1\frac{3}{4}$ or $2\frac{1}{2}$, they really were in agreement except in the single instance of Gartons at Winnipeg. In other words, with a single exception among 12 comparisons the statement that the optimum rate for fertilizer and no fertilizer treatments was the same, held regardless of variety.

The analyses of variance for this interaction support the indicated conclusions since the $V \times R \times F$ mean squares are not significant at any of the stations.

SUMMARY AND CONCLUSIONS

Results of experiments involving fertilizer treatment and rates of seeding with barley are reported. Two kinds of fertilizer were used namely 16-20-0 applied at the rate of 96 pounds per acre and 11-48-0 at 40 pounds per acre. The application was made by the drill method at the time of seeding. The rates of seeding were 1 bushel, $1\frac{3}{4}$ bushels and $2\frac{1}{2}$ bushels per acre. The three varieties used were O.A.C. 21, Gartons, and Mensury Ottawa 60. The tests were conducted at each of four stations namely Winnipeg, Carman, Newdale and Swan River.

The differences between the two kinds of fertilizer, although consistent, were too small to be of practical consequence.

Averaging the data for both fertilizers it was found that fertilizer treatment increased yields by amounts ranging from 2 bushels per acre at Winnipeg to 11.0 bushels at Swan River. In relation to the total yields in each case the increases were large at Swan River and Carman, considerable at Newdale and small at Winnipeg. The differences were highly significant according to statistical analysis.

The variety Gartons responded much less to fertilizer treatment than the other two varieties at the three stations Winnipeg, Newdale and Swan River. The lower response was significant at those stations. Although reduced response was also indicated at Carman, it was not significant.

The greatest response to fertilizer treatment took place at the most favourable seeding date at all of the stations. This differential response was significant only at Swan River and Carman.

Analysis of the triple interaction between variety, fertilizer and date of seeding disclosed that it was not significant at any of the stations.

The seeding rate $1\frac{3}{4}$ bushels per acre resulted in a substantial increase in yield over that for the 1 bushel rate. Only a small further increase resulted from the $2\frac{1}{2}$ bushel rate.

Gartons showed less response to increased seeding rate than the other two varieties. The behaviour was consistent at all stations, but was statistically significant only at Winnipeg and Newdale.

In general the order of yield under different rates of seeding was consistent at all dates, although at Winnipeg the increase due to increased rate was distinctly greater at the second and third or less favourable dates than at the first. This differential response was significant at that station.

There was no interaction between rate of seeding and fertilizer treatment.

There was no triple interaction between rate of seeding, fertilizer treatment and variety. In other words, with insignificant exceptions, the same rate of seeding was optimum under all fertilizer treatments for all varieties.

APPENDIX

Appendix Table 11.—Analyses of variance

Variance due to	Winnipeg		Carman		Newdale		Swan River	
	D.F.	Mean Squares	D.F.	Mean Squares	D.F.	Mean Squares	D.F.	Mean Squares
Blocks	72	171.5	48	155.8	48	57.1	48	332.7
Varieties	2	656.5	2	1954.8	2	86.3	2	2939.0
Rates	2	1519.6	2	843.3	2	196.6	2	1600.5
Fertilizer	2	373.7	2	2345.2	2	320.7	2	6620.2
Dates	2*	16729.6	1*	24537.6	2*	753.5	2*	984.7
Years	2	14985.6	2	12517.4	1	2591.5	1	14052.0
V × R	4	348.7	4	143.8	4	70.2	4	205.8
V × F	4	173.0	4	129.7	4	38.6	4	411.1
R × F	4	104.3	4	27.8	4	14.8	4	24.5
V × R × F	6	4.2	6	45.4	6	11.8	6	48.2
V × D	4	3024.8	2	2.0	4	215.6	4	1239.7
R × D	4	248.7	2	80.1	4	10.0	4	69.5
F × D	4	78.6	2	373.9	4	19.4	4	935.3
V × R × D	8	79.2	4	118.4	8	12.0	8	44.4
V × F × D	8	32.1	4	18.0	8	11.9	8	30.4
R × F × D	8	64.0	4	46.4	8	24.9	8	19.0
V × R × F × D	12	48.5	6	15.0	12	15.6	12	52.2
D × Y	4	2809.6	2	1675.0	2	382.2	2	4132.5
V × Y	4	3244.1	4	447.4	2	241.6	2	2681.5
R × Y	4	15.4	4	1415.5	2	17.4	2	112.7
F × Y	4	53.4	4	184.0	2	56.5	2	1303.5
V × R × Y	8	77.2	8	94.0	4	14.7	4	47.5
V × F × Y	8	22.7	8	29.5	4	19.2	4	144.8
R × F × Y	8	24.5	8	39.3	4	6.2	4	6.7
V × R × F × Y	12	34.3	12	43.9	6	15.6	6	109.9
V × D × Y	8	653.5	4	125.2	4	107.0	4	2255.0
R × D × Y	8	131.0	4	43.1	4	47.9	4	12.2
F × D × Y	8	137.1	4	404.0	4	98.7	4	161.4
V × R × D × Y	16	60.6	8	64.8	8	18.2	8	58.6
V × F × D × Y	16	105.2	8	42.0	8	21.9	8	60.8
R × F × D × Y	16	48.6	8	31.4	8	19.7	8	69.5
V × R × F × D × Y	24	63.4	12	263.0	12	7.3	12	24.7
Error	432	32.3	288	9.5	288	18.0	288	30.6

* Not comparable.

Appendix Table 12.—Effect of fertilizer application upon the yields of 3 varieties of barley sown at 3 rates per acre

Station and variety	1 bushel		1¾ bushels		2½ bushels		Mean (all rates)	
	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.
Winnipeg								
O.A.C. 21	35.3	30.3	39.4	35.4	40.5	39.3	38.4	35.0
Gartons	39.4	38.9	39.2	39.0	38.8	41.4	39.1	39.8
Mensury	32.8	29.1	39.0	35.2	39.9	37.4	37.2	33.9
Mean	35.8	32.8	39.3	36.5	39.7	39.4	38.2	36.2
Carman								
O.A.C. 21	26.4	17.0	30.0	23.7	32.6	23.6	29.7	21.4
Gartons	36.6	29.9	36.8	32.9	37.0	32.7	36.8	31.8
Mensury	22.9	16.6	27.1	17.1	29.9	23.9	26.6	19.2
Mean	28.6	21.2	31.3	24.6	33.2	26.7	31.0	24.1
Newdale								
O.A.C. 21	21.0	19.7	23.1	19.8	26.1	20.8	23.4	20.1
Gartons	24.0	23.0	24.8	23.8	25.5	23.4	24.8	23.4
Mensury	19.4	16.0	24.1	18.8	24.4	20.7	22.6	18.5
Mean	21.5	19.5	24.0	20.8	25.3	21.6	23.6	20.6
Swan River								
O.A.C. 21	45.3	27.8	49.8	40.4	52.4	41.2	49.2	36.5
Gartons	50.0	46.9	55.1	47.5	53.6	47.2	52.9	47.2
Mensury	44.7	30.0	46.6	34.5	51.9	34.6	47.7	33.0
Mean	46.7	34.9	50.5	40.8	52.6	41.0	49.9	38.9

Appendix Table 13.—Effect of fertilizer applied to barley when sown on 3 successive dates at 3 different rates per acre (means over all varieties)

Variety and station	1 bushel		1¾ bushels		2½ bushels		Mean (all rates)	
	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.
Winnipeg								
Date 1	46.2	41.5	46.9	42.5	46.2	44.3	46.4	42.8
Date 2	35.8	32.4	40.2	39.6	41.5	42.2	39.2	38.1
Date 3	25.5	24.4	30.5	27.5	31.6	31.5	29.2	27.8
Mean	35.8	32.8	39.2	36.5	39.8	39.3	38.2	36.2
Carman								
Date 1	42.1	27.9	44.7	36.0	48.5	37.8	45.1	33.9
Date 2	28.9	24.6	31.5	26.5	32.0	28.9	30.8	26.7
Date 3	14.9	11.0	17.7	11.2	19.1	13.5	17.2	11.9
Mean	28.6	21.2	31.3	24.6	33.2	26.7	31.0	24.2
Newdale								
Date 1	24.1	21.8	26.8	22.7	28.9	23.2	26.6	22.5
Date 2	21.4	20.2	24.3	21.8	25.4	23.3	23.7	21.8
Date 3	18.9	16.7	20.9	17.9	21.7	18.4	20.5	17.7
Mean	21.5	19.5	24.0	20.8	25.3	21.6	23.6	20.6
Swan River								
Date 1	44.9	32.5	48.9	37.9	49.5	37.0	47.8	35.8
Date 2	50.1	32.5	55.9	39.8	57.8	38.8	54.6	37.0
Date 3	44.9	39.7	46.8	44.7	50.5	47.2	47.4	43.9
Mean	46.6	34.9	50.5	40.8	52.6	41.0	49.9	38.9

APPENDIX TABLE 14.—EFFECT OF FERTILIZER UPON THE YIELD OF 3 VARIETIES OF BARLEY SOWN AT 3 SUCCESSIVE DATES

Station and variety	Date 1		Date 2		Date 3		Mean (all dates)	
	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.	Fert.	No fert.
Winnipeg								
O.A.C. 21	50.1	43.5	40.5	38.6	24.7	22.9	38.4	35.0
Gartons	41.1	40.2	38.5	40.5	37.9	38.8	39.1	39.8
Mensury	48.0	44.7	38.5	35.2	25.1	21.8	37.2	33.9
Mean	46.4	42.8	39.2	38.1	29.2	27.8	38.2	36.2
Carman								
O.A.C. 21	45.2	31.0	29.3	24.8	14.5	8.4	29.7	21.4
Gartons	48.1	41.4	36.7	33.9	25.6	20.3	36.8	31.8
Mensury	41.9	29.2	26.4	21.3	11.5	7.0	26.6	19.2
Mean	45.1	33.9	30.8	26.7	17.2	11.9	31.0	24.2
Newdale								
O.A.C. 21	27.3	22.5	23.3	21.2	19.6	16.7	23.4	20.1
Gartons	25.3	23.0	25.8	25.1	23.2	22.1	24.8	23.4
Mensury	27.1	22.1	22.0	19.0	18.7	14.2	22.6	18.5
Mean	26.6	22.5	23.7	21.8	20.5	17.7	23.6	20.6
Swan River								
O.A.C. 21	48.1	34.7	54.8	35.5	44.5	39.2	49.2	36.5
Gartons	46.2	40.7	55.6	43.6	57.0	57.3	52.9	47.2
Mensury	49.2	32.1	53.4	32.0	40.7	35.1	47.7	33.0
Mean	47.8	35.8	54.6	37.0	47.4	43.9	49.9	38.9

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PYTHIUM ROOT ROT OF GRASSES[1]

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[Received for publication January 29, 1942]

INTRODUCTION

In recent years the value of grasses in prairie agriculture has been stressed and an increase in acreage urged by qualified agricultural workers both in published papers and on public platforms. Partly because of this and also because of present economic conditions and demands there has been, in the last few years, an increase in the grass acreage for hay and seed, and in seeded pasture. It seems timely that attention should be drawn to the importance of grass diseases and to their possible effects on the succeeding crops in the rotation. This paper deals with *Pythium* root rot of grasses and may be considered in many ways supplementary to previously published work by Vanterpool and co-workers (20 to 24, 26) on browning root rot of cereals (*Pythium* spp.) which is one of the major crop diseases in Saskatchewan. The disease on grasses is not as characteristic and clear-cut in its symptoms as it is on cereals, so that the name browning root rot of grasses is not entirely appropriate. In this paper the designation "*Pythium* root rot of grasses" will be used.

Early in our investigations on browning root rot of cereals, it was realized that the same *Pythium* spp. causing this disease were also pathogenic on grasses. This was well demonstrated in an experiment conducted in 1930 (Figures 1 and 2) which showed the effects of strains of *Pythium arrhenomanes* Drech. from sugarcane, maize and wheat on the growth of brome (*Bromus inermis* Leyss.) and slender wheat grass (*Agropyron pauciflorum* Hitchc.) grown in sterilized soil containing sufficient inoculum to produce moderate damage. Hence the importance of grasses in relation to the disease on cereals was pointed out from time to time especially in control recommendations (22, 24).

The double aspect of the *Pythium* disease of grasses just referred to makes it particularly difficult to assess accurately the direct and indirect damage caused, but it is safe to say that the total losses are higher than has been generally realized.

REVIEW OF LITERATURE

Root diseases of grasses, largely because of their very nature, have not until recently received the attention which their importance warrants. The first reports of *Pythium* damage to grasses concerned the killing of turf on lawns and golf courses (11, 3, 19, 1, 12). Monteith and Dahl (11), in 1932, were among the first to report on *Pythium* attacking turf in the United States. They describe a spotlight disease of turf (*Agrostis* spp.) on golf courses caused by *P. butleri* Subr., and state that it is most likely to be destructive on new rather than on established turf. Other investigators have since isolated *Pythium* spp. from damaged grass turf on lawns

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FIGURE 1. Brome grass grown in sterilized soil inoculated with various strains of *Pythium arrhenomanes*. Pot *1*, sugar-cane strain; *2*, maize strain; *3*, wheat strain 10; *4*, wheat strain 18; and *5*, control.

FIGURE 2. Same as Figure 1, but showing the effects on slender wheat grass.

or golf courses and have shown that the species concerned could infest various grasses when artificially inoculated (3, 19, 1, 12, *cf.* 9). In 1933, a species of *Pythium* was found to be causing serious damage to a lawn of crested wheat grass (*Agropyron cristatum* (L.) Beauv.) at Winnipeg, Canada (3). This report was of particular interest, as crested wheat is one of the most important hay and pasture grasses on the prairies. In the following year, Vanterpool (4), in Saskatchewan, obtained *P. arrhenomanes* from eight grasses grown in field soil naturally infested with *Pythium* spp. causing browning root rot of wheat. *Pythium* oospores were observed in the roots of three other grasses, and in a later note (5) additional grass hosts were listed. The same author (21), in 1938, stated that *P. aristosporum* Vant. and *P. tardicrescens* Vant., two of the fungi capable of causing browning root rot of wheat, were also capable of attacking grasses. Ho and Melhus (8), in 1938, in the United States, found that *P. graminicola* Subr. isolated from barley was capable of infecting timothy (*Phleum pratense* L.). Vanterpool (6), in 1939, found couch grass (*Agropyron repens* (L.) Beauv.) and green foxtail (*Setaria viridis* (L.) Beauv.), a very susceptible grass, to be attacked in the field. Sprague (13), in 1940, in North Dakota, isolated *P. arrhenomanes* from various grasses and found it to be particularly common on pigeon grass (*Setaria viridis* (L.) Beauv.). During the early summer of 1941, the present writer isolated strains of *P. aristosporum*, *P. arrhenomanes* and *P. graminicola* from lesioned roots of brome, crested wheat and slender wheat grasses growing on farms in north-central Saskatchewan. All of these species proved to be highly pathogenic to wheat seedlings, producing a severe, brown necrosis of the roots. Strains of *P. debaryanum* Hesse and close allies, obtained at the same time, were slightly to moderately pathogenic to wheat under the same conditions, causing a stunting of the roots and increased root-hair production behind

the root tip, which became slightly discoloured. From a recent communication by Vanterpool and Sprague (25) it appears that *Pythium* damage to cereals and grasses is as common and serious in North Dakota and adjoining states as in Saskatchewan.

PYTHIUM DAMAGE TO GRASSES

There appear to be four types or phases of *Pythium* damage to grasses. The first is a pre-emergence killing of the seedlings in which both roots and shoots are attacked; the second consists of damping-off or early seedling killing due to the rotting of the roots and the bases of the stems; the third is a lesioning of some of the coarser roots and an invasion of many of the fine laterals in the late seedling stage while the plants are still becoming established; and the fourth consists of a lesioning of the new batches of roots produced on perennial grasses during subsequent growth periods depending on the type of re-rooting of the grass variety (17). The first two types result in a reduction in stand, and from general observation one might assume that these types are the most serious, but the damage in the aggregate caused by the third and fourth types, though more difficult to assess, may be considerable. In comparison, the pre-emergence and damping-off types rarely occur on cereals in Saskatchewan.

Pathogenic species of *Pythium* are most readily isolated from diseased roots of young plants up to the third week of June; the majority of these are lobulate sporangial forms and the same species which cause browning root rot of cereals. In very young seedlings they are more commonly obtained from the stem bases than is the case with cereals. With the approach of hot weather, fewer of these forms are obtained, while more slightly and moderately pathogenic and non-pathogenic sphaerosporangial forms appear. Virulent species are not easily obtained in culture from the older roots of grass plots two or more years old. Indeed, oospores are not commonly found in such roots. Thus, in preliminary attempts to isolate *Pythium* species from native grasses in virgin prairie, no virulent forms were obtained; but when cereals and grasses are grown in virgin soil in pots, their young roots usually show *Pythium* lesioning, and the usual array of pathogenic species can readily be isolated (24).

All the cultivated grasses in Saskatchewan are attacked. Millet (*Setaria italica* (L.) Beauv.) and Sorghum are very susceptible and will probably not do well as annual hay crops in areas where *Pythium* root rot is known to be common. The yield will of course be reduced, and there is the likelihood that soil infestation of the root-destroying fungi may be increased.

EXPERIMENTAL

The soil used in these experiments was collected in the June preceding the date of the experiment from wheat fields heavily attacked by browning (*Pythium*) root rot. All samples were taken to spade depth, the *Pythium*-infested soil from the browning root-rot areas, and the normal soil from areas with apparently healthy plants. The dry weight data given in the tables represent the dry weights of the entire plants, the roots having been carefully washed free of soil before drying.

Experiment I. Growth of grasses and non-grasses in *Pythium*-infested and normal field soils.

Separate sowings of each crop plant were made one-half to three-quarters of an inch deep in 6-inch pots at the rate of 50 seeds to the pot for the grasses, and 33 seeds for the non-grasses. The pots were placed on the greenhouse bench and the soil kept reasonably moist. Over-head supplementary illumination was supplied for a few hours from about 4 p.m. during periods of dull weather. The results are presented in Table 1.

TABLE 1.—COMPARATIVE YIELDS OF VARIOUS CROPS IN NORMAL AND *Pythium*-INFESTED FIELD SOILS IN THE GREENHOUSE

Crop	Normal field soil		Pythium-infested field soil		Infested as a percentage of normal
	Germination	Dry weight	Germination	Dry weight	
	%	*gm.*	%	*gm.*	%
Experiment Ia*					
Brome grass		10.05		5.78	57.5
Crested wheat grass		7.21		3.38	46.9
Slender wheat grass		7.40		6.13	82.9
Alfalfa (Grimm)		4.55		3.03	66.6
Sweet clover		6.85		6.93	101.2
Flax (Bison)		7.45		6.92	92.9
Experiment Ib†					
Brome grass	86	12.71	87	9.69	76.2
Crested wheat grass	89	13.11	82	7.65	58.4
Slender wheat grass	66	8.12	67	3.65	44.9
Alfalfa (Grimm)	46	5.33	42	3.02	56.7
Sweet clover	84	8.67	78	7.11	82.0
Flax (Bison)	91	6.68	96	7.91	118.4

* October 5 to December 23, 1940. Two replicates to each treatment.
† February 3 to March 31, 1941. Four replicates to each treatment.

Both Figure 3 and the comparative results in Table 1 show that there is an appreciable reduction in yield of grasses in the *Pythium*-infested soil compared with normal field soil. The low yield as well as the large reduction in yield of alfalfa (*Medicago sativa* L.) may possibly be due to the poor

FIGURE 3. From left to right: Paired pots of brome, Fairway crested wheat and slender wheat grasses, with natural *Pythium*-infested soil on the left, and normal field soil on the right, of each pair.

seed resulting in weakened seeedlings less able to withstand damping-off fungi of the *P. debaryanum* type. Sweet clover (*Melilotus alba* Desr.) and flax (*Linum usitatissimum* L.), on the contrary, show no evidence of being consistently affected. Usually all non-grass crops show some reduction in yield in the infested soil owing to phosphate deficiency (20, 23, 24), and not to the presence of lobulate *Pythium* spp. which are readily isolated from diseased grass roots, but never from the roots of the legumes or flax. However, isolates of *P. debaryanum* and close allies can be obtained from the roots of legumes and flax though the roots of these plants do not show signs of severe, brown, necrotic lesioning so characteristic of grass and cereal roots in infested field soil.

Experiment II. Growth of grasses in *Pythium*-infested soil, steam-sterilized infested soil, and normal soil of different moisture contents.

Brome, crested wheat and slender wheat grasses were grown in 1-gallon galvanized-iron cans (coated on the inside with asphalt paint) in soil maintained at 30 and 60% respectively of the moisture-holding capacity. There were 50 seeds to each can, with all varieties sown in duplicate.

TABLE 2.—EFFECT OF MOISTURE CONTENT ON THE GROWTH OF GRASSES IN INFESTED, STEAM-STERILIZED INFESTED, AND NORMAL FIELD SOILS IN POT EXPERIMENTS
JANUARY 27 TO MARCH 31, 1941

Grass	Percentage m.h.c.* of soil	Germination			Dry Weight		
		Pythium-infested field soil	Steam-sterilized infested soil	Normal field soil	Pythium-infested field soil	Steam-sterilized infested soil	Normal field soil
	%	%	%	%	gm.	gm.	gm.
Brome (Commercial)	30	88	95	—	4.72	13.48	—
	60	77	96	—	11.40	21.58	—
Crested wheat (Fairway)	30	77	87	71	3.80	14.74	9.23
	60	65	92	76	10.10	19.05	14.23
Slender wheat	30	41	52	58	2.82	7.92	8.15
	60	68	72	61	11.25	17.71	11.92
Averages	30	68.6	78.0	64.5	3.78	12.05	8.69
	60	70.0	86.6	68.5	10.92	19.45	13.08

* Moisture-holding capacity.

The large increase in the growth and germination (Table 2) in the steam-sterilized soil over the infested soil is due to the increase in nutrients and the destruction of root-destroying fungi in the steamed soil (Figures 4 and 5). The results indicate that the factors limiting yield are more effective in the "dry" soil than in the "moist." Thus, taking the average for the three grasses, the yield in the dry, infested soil is only 31.3% of that in the dry, sterilized soil, while the yield in the moist, infested soil is 56.1% of that in the moist, sterilized soil. Also, in the infested soil, the average yield under the dry conditions is 34.6% of that under the moist conditions, whereas in the sterilized soil the ratio for the corresponding

FIGURE 4. From left to right: Fairway crested wheat grass in normal, naturally infested, and steam-sterilized infested soil.

FIGURE 5. As in Figure 4, showing slender wheat grass under the same conditions.

moistures is 61.4%. Unpublished data of temperature-tank studies indicate also that pre-emergence killing and early seedling damage or damping-off of crested wheat grass are greater at 24° C. than at 14° C. in dry soil of 30% moisture-holding capacity. Stevenson and Kirk (15) have stated that the seedlings of crested wheat grass "may be severely injured by dry, hot weather, although when once established the plants are extremely drought resistant." The evidence cited above suggests that the detrimental effects of dry, hot weather may not be due entirely to these factors acting directly on the grass, but partly to the effects of root-rotting soil fungi under these particular conditions. This question is of interest and needs further elucidation.

Experiment III. Effect of fertilizers on grasses in *Pythium*-infested soil under greenhouse conditions.

It has been shown experimentally and from field observations that phosphatic fertilizers greatly increase the yield of wheat in browning (*Pythium*-infested) soil and that they may be used as a practical control measure (20, 23, 24). The 11–48[3] ammonium phosphate proved to be one of the best. In Experiment IIIa, with brome, crested wheat and slender wheat grasses, this fertilizer was incorporated in the top two inches of soil in 6-inch pots at a rate equivalent to 200 pounds per acre, just before sowing. The experiment was conducted in triplicate. The results are given in Table 3.

The results show definitely that significant, early increases in the growth of grasses in browning soil in pot experiments are obtained when ammonium phosphate is added as an amendment (Figure 6).

[3] Available nitrogen 11% and available phosphorus pentoxide 48%.

6

7

8

FIGURE 6. The effect of ammonium phosphate (11-48) on various grasses in naturally infested soil. From left to right: Paired pots of brome, Fairway crested wheat and slender wheat grasses, with the untreated pot on the left and the fertilized on the right of each pair.

FIGURE 7. The effect of various fertilizers on Fairway crested wheat grass in naturally infested soil. Left to right: Untreated, triple superphosphate, ammonium phosphate (11-48), ammonium phosphate (16-20), and ammonium sulphate.

FIGURE 8. As in Figure 7, but in normal field soil.

TABLE 3.—THE EFFECT OF AMMONIUM PHOSPHATE ON GRASSES IN *Pythium*-INFESTED FIELD SOIL IN POT EXPERIMENTS. EXPERIMENT IIIA, FEBRUARY 17 TO MARCH 31, 1941

Treatment	Brome		Crested wheat		Slender wheat	
	Dry weight	Increase	Dry weight	Increase	Dry weight	Increase
	gm.	%	*gm.*	%	*gm.*	%
Untreated	7.8	—	6.5	—	5.1	—
Ammonium phosphate (11–48)	18.3	134.6	14.4	121.5	15.3	200.0

Experiment IIIb was conducted in a manner similar to that of IIIa, each fertilizer being applied at a rate equivalent to 200 pounds per acre to its respective pots. That part of the experiment in which "stubble" soil was used, was not run contemporaneously with the main part of the experiment. The results are, nevertheless, of comparative value and have accordingly been included in Table 4.

TABLE 4.—THE EFFECT OF VARIOUS FERTILIZERS ON FAIRWAY CRESTED WHEAT GRASS IN *Pythium*-INFESTED AND NORMAL FIELD SOIL IN POT EXPERIMENTS. EXPERIMENT IIIB

Treatment*	April 7 to May 19, 1941				May 12 to July 23	
	Infested soil		Normal soil		Infested "Stubble†" soil	
	Dry weight	Increase	Dry weight	Increase	Dry weight	Increase
	gm.	%	*gm.*	%	*gm.*	%
Untreated	7.6	—	10.0	—	6.7	—
Triple superphosphate (0–43)	15.4	102.6	15.9	59.0	10.0	49.2
Ammonium phosphate (11–48)	19.7	159.2	22.6	126.0	15.5	131.3
Ammonium phosphate (16–20)	18.6	144.7	21.0	110.0	15.1	125.3
Ammonium sulphate (20–0–0)	7.2	− 5.3	15.8	58.0	8.7	29.8

* Four replicates for each treatment.
† Infested "stubble" soil is infested soil which has borne a crop of wheat in the greenhouse and may be considered more or less comparable to soil collected from an infested area after harvest.

Experiment IIIc is similar to that part of Experiment IIIb in which infested and normal soils were used. It was, however, conducted during the winter months when light conditions were unfavourable. More reliance, therefore, should be placed on the results of Experiment IIIb, Table 4.

The results in Tables 4 and 5 should be compared closely with those showing the effects of the same fertilizers on the disease in wheat under similar conditions (23, Table 1 and Figures 1 and 2; 20). They show that the grasses respond relatively better to triple superphosphate (0–43) in

TABLE 5.—THE EFFECT OF VARIOUS FERTILIZERS ON BROME AND FAIRWAY CRESTED WHEAT GRASSES IN *Pythium*-INFESTED AND NORMAL FIELD SOIL IN POT EXPERIMENTS. EXPERIMENT IIIc, OCTOBER 18 TO DECEMBER 15, 1941

Treatment*	Infested soil			Normal soil		
	Germ-ination	Dry weight	In-crease	Germ-ination	Dry weight	In-crease
	%	*gm.*	%	%	*gm.*	%
Brome:						
Untreated	87	4.10	—	81	6.75	—
Triple superphosphate (0–43)	77	6.10	48.8	82	7.20	6.6
Ammonium phosphate (11-48)	85	6.70	63.4	78	8.25	22.2
Ammonium phosphate (16-20)	84	7.30	78.0	70	8.20	21.5
Ammonium sulphate (20-0-0)	79	3.87	− 5.6	81	6.90	2.2
Crested Wheat:						
Untreated	74	1.00	—	76	2.87	—
Triple superphosphate (0-43)	69	2.08	108.0	73	3.40	18.4
Ammonium phosphate (11-48)	70	2.00	100.0	82	3.80	32.4
Ammonium phosphate (16-20)	70	1.75	75.0	74	2.89	0.6
Ammonium sulphate (20-0-0)	66	1.45	45.0	74	2.20	−23.3

* Two replicates for each treatment.

naturally infested than in normal soil. The same is true, though to a less degree, of the two ammonium phosphates. These results were to be expected in view of the known lower available phosphorus content of the infested soil (20, 23, 24). The fact that the two ammonium phosphates usually give the highest increase in yield indicates that nitrogen is also deficient in these soils. That nitrogen is not the limiting element, however, is indicated by the negative responses of crested wheat grass in Experiment IIIb, and brome in Experiment IIIc to ammonium sulphate in the infested soil. No reason is known for the unexpected positive response in Experiment IIIc of crested wheat grass to ammonium sulphate in infested soil. It would seem unwise to apply ammonium sulphate alone to these soils at the time of seeding, especially if this be done on fallowed land. As in the experiment with wheat, phosphorus is shown to be the limiting element, but once its deficiency is made good, added nitrogen will give further response (23). As normal soil usually contains slightly less nitrate than infested soil when collections are made in June soon after symptoms have appeared (20, 23), a slight response by the normal soil to ammonium sulphate, as occurred in Experiment IIIb, might be expected. This expectation, however, was not borne out in Experiment IIIc.

Similarly, with the "stubble" soil, the relatively low response to the triple superphosphate is probably limited by the deficiency of nitrate, and

conversely, the low response to the ammonium sulphate is limited by the low phosphorus. Good responses are obtained from the fertilizers containing both nitrogen and phosphorus.

All these results of the effects of the various fertilizers on grasses closely parallel those obtained on wheat (20, 23).

OTHER WORK STILL IN PROGRESS

1. *The Effect of the Preceding Crop on Browning Root Rot of Wheat*

It is very desirable practically to know what effect the preceding crop in the rotation has on the incidence of *Pythium* root rot in wheat. It is admittedly not possible to produce conditions in a rotation experiment in the greenhouse closely approaching those pertaining in the field. Such experiments may, however, pave the way for field experiments and help in the interpretation of the results and of observations on farm crops. Some experiments on this problem have been completed and others are in progress. It may tentatively be inferred from the results that though the yield of wheat after grasses is usually lower than after legumes and flax, the number of *Pythium* lesions on wheat roots following grasses is usually no greater than after non-grasses. The decreased growth is doubtless due to a deficiency in nitrate. Thus there is no evidence of any definite increase in *Pythium* inoculum in a virulent form in the soil by the grasses used, namely, brome, crested wheat and slender wheat grasses. A possible explanation is that the decomposition of the added organic matter brings about a microbiological and nutrient balance which keeps the pathogenic *Pythium* spp. in abeyance. The condition is probably analogous to that prevailing in the wheat crop on stubble in which the disease is rarely of any concern (24). Until evidence to the contrary is available, it may be suggested that when choosing grasses preference be given to the strongly rooting grasses, namely, brome and crested wheat (*cf.* 16) in areas where browning root rot of wheat is severe. This is in line with agronomic recommendations (16).

2. *Effect of Seed Treatment*

Grass seed in several different experiments has been dusted with Ceresan and Spergon disinfectants and sown in naturally infested soil in flats 16 × 12 × 4 inches deep. Usually, with a few exceptions, there was a slight increase in stand with both disinfectants, indicating that the germinating seedling was given some protection from the damping-off fungi present in the soil and probably also from seed-borne pathogens. This initial gain was not consistently reflected in increased dry weight after about two months. There may be conditions relating to both seed and field in which disinfection would be beneficial and worth trying, but until further work has been done, seed treatment of grasses for the reduction of *Pythium* root rot in the field is not recommended. It may, however, prove helpful in greenhouse experimental work with grasses, especially if the germinability be low.

DISCUSSION

The *Pythium* species parasitic on the roots of graminaceous hosts are indigenous soil-inhabiting fungi which are widespread in Saskatchewan soils. They are not seed-borne. Until such a time as highly resistant

strains of commercial grasses are obtained, reduction in the damage due to these fungi must be brought about by the use of high grade seed of good germinability likely to produce strong seedlings capable of withstanding the fungus attacks, and also by manipulation of the soil environment. The recent suggestion of Aamodt, Lefebvre and Johnson (2) that the production of disease-resistant strains by selection and hybridization appears to be the only logical method of controlling certain diseases of forage grasses is an important one, and applies particularly well to *Pythium* root rot. Already strains of milo (*Sorghum* sp.) resistant to root rot caused by *P. arrhenomanes* have been secured in Kansas (27) and California (10).

The damage to both turf and grasses in the field appears to be worst during the first year, especially during the early establishment phase, but the batches of new roots formed during subsequent growth phases are also liable to attack. Thus it may be, at least under field conditions, that the resistance of a given variety may vary at these two different stages of growth. It is possible that such a condition might help to explain why slender wheat grass compares well with brome and crested wheat grass during the first year or two, but does relatively worse in succeeding years. Another factor which should be considered in relation to the root rot is whether the grass is an "annual" or a "perennial" rooter according to the recent classification of Stuckey (15).

The disease on turf can be more easily recognized than the same disease on crop grasses and treated directly by the application of a suitable fungicide and appropriate conditions of watering (11).

Pythium root rot is more severe at higher than at lower temperatures. This may explain why June seedings of certain grasses, such as crested wheat which is a cool temperature plant, are more liable to early seedling killing than spring or fall seedings (15). Two facts suggest that the fall planting may prove more beneficial than the spring in limiting root-rot injury; these are, first, that relatively high temperatures are more likely to occur before the spring seedlings become established, and second, that since it is generally recommended by agronomists that fall plantings of grasses be done on stubble, they are less likely to be affected by the root rot than if sown on fallow, that is, if grasses respond to the disease in a manner similar to wheat, which is little affected on stubble (24).

The high increase in yields of grasses obtained when phosphatic fertilizers are applied to naturally infested field soil, suggests that these fertilizers be applied with the grass seed, in experimental strips at least, on fields where browning root rot of wheat is known to have been severe, and where economical increases in yield of wheat have been obtained from the use of such fertilizers. This practice would appear to be best with a spring seeding of grasses on fallowed land, but under the same conditions ammonium sulphate alone should not be applied to these phosphorus-deficient soils.

Whether the application of phosphatic fertilizers proves economically beneficial or not depends largely on the percentage of the field which was attacked by browning root rot and therefore deficient in phosphorus. It

might be argued that though the increase in yield may not be directly economical, the improved quality of the hay, which is a particularly important point in mixed farming, would be considerably enhanced. Of interest here is the report of Theiler, Green and Du Toit (18) of a disease in cattle in South Africa caused by phosphorus starvation resulting from feeding grasses grown on soil deficient in phosphorus. They found the phosphorus content of the old grass particularly to be extraordinarily low. Among investigations on this problem in North America may be cited some work done in Minnesota by Eckles, Gullickson and Palmer (7). They clearly demonstrated the correlation of certain disease symptoms in cattle with the low phosphorus content of the grasses fed to them and with the low phosphorus content of the soils on which the grasses were grown. They showed that the phosphorus content of the forage plants was low in years of lower rainfall, and also that the phosphorus content of prairie hay and timothy was roughly half of that in alfalfa hay. The application of ammonium phosphate to sod of brome and other grasses in early spring may prove advantageous in browning districts in Saskatchewan.

Grass-legume mixtures would seem to be a means of providing a restraining influence on *Pythium* root rot of grasses and on the succeeding cereal crop, and at the same time of supplying a forage of good feeding quality. The legume is not susceptible to the *Pythium* spp. severely pathogenic to grass roots, and it is also advantageous in many other respects, as pointed out by Stevenson (14).

The question of the fertilizer requirements of grasses in Saskatchewan might well be reconsidered or given reassessment at this time in certain localities in view of root-disease problems and the increase in grass growing and mixed farming. The whole problem of root rots of grasses is a complex one and a great deal of work remains to be done.

SUMMARY

This paper deals with some studies on *Pythium* root rot of grasses conducted primarily as supplementary to the more important browning root rot (*Pythium* spp.) on cereals.

The common commercial grasses, when grown in the greenhouse in *Pythium*-infested soil collected from areas in wheat fields severely diseased with browning root rot, themselves become heavily attacked. The same pathogenic species of *Pythium*, namely, *P. aristosporum* Vant., *P. arrhenomanes* Drech., *P. graminicola* Subr., *P. tardicrescens* Vant. and *P. volutum* Vant. & Trusc., are of major concern on both grasses and cereals, with *P. debaryanum* Hesse and close allies showing less pathogenic propensities. Under the same conditions legume and flax roots are not attacked by the forms severely pathogenic on graminaceous hosts, but forms of the *P. debaryanum* group may be isolated, though the roots commonly show little lesioning. The damage to grasses in the field appears to be greatest in the early stages of growth when the plants are becoming established. This suggests the use of high grade seed as an initial step in disease control. Further damage may be done in subsequent growth periods when new roots are emerging from the crown.

In these infested field soils, phosphate-containing fertilizers, especially ammonium phosphate (11–48), are found to increase the growth of grasses considerably in pot experiments when applied at the time of seeding. Ammonium sulphate alone, under the same conditions, usually has no effect or may be slightly detrimental. The effects of the various fertilizers on grasses in normal field soil during the establishment phase are also given.

It is tentatively suggested that if grasses are to be grown on fields on which browning root rot of wheat has been severe and which have given economical increases in yield from phosphatic fertilizer amendments, that at least trial strip applications of ammonium phosphate be made, as it is believed that more vigorous seedlings and a better stand will be secured in such areas, especially if the seeding be done on fallowed land. An increase in the quality or nutritive value of the forage will also result.

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OBSERVATIONS ON ANTAGONISM IN INOCULATION TESTS OF WHEAT WITH *HELMINTHOSPORIUM SATIVUM* P.K. & B., AND *FUSARIUM CULMORUM* (W. G. SM.) SACC.[1]

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[Received for publication February 12, 1942]

INTRODUCTION

In the field of soil microbiology, in recent years there has been a good deal of attention directed toward association effects of the microflora. Much of the work done hinges on attempts at biologic control of pathogenic organisms. In numerous cases such control, induced by the introduction of an organism or organisms antagonistic to the pathogen has been to a certain degree successful. Moreover, the interactions, inhibitory or otherwise between various pathogens must be considered, especially in work involving inoculation studies. Problems of this nature frequently arise in cereal root disease studies. In the past variable results were obtained in inoculation attempts with rootrot fungi. This was generally attributed to inability to control all the environmental factors. Studies on moisture, temperature and other relations partially solved the problem. Recently the part played by the soil microflora in influencing these fungi has also been demonstrated. It has been shown that certain members of the microflora exert varying degrees of control on *Ophiobolus graminis* Sacc. (15). Henry (8) found the same to be true in the case of *Helminthosporium sativum* P.K. and B. *Cephalothecium roseum* Corda was responsible for reducing the pathogenicity of *H. sativum* in pot culture according to Greaney and Machacek (6). In dealing with *Trichoderma lignorum* (Tode) Harz., Bisby James and Timonin (1) showed that it suppressed the virulence of *H. sativum* and *Fusarium culmorum* (W.G.Sm.) Sacc. in pot tests and Haenseler and Allen (7) demonstrated that by inoculating the soil with *Trichoderma* "damping off" of cucumber seedlings could be controlled. Sanford (14) states that a strain of bacteria isolated from the soil inhibits the development of *Actinomyces scabies* (Thaxt.) Güssow causing potato scab. Control of this disease by green manuring has been attributed to antagonism by Millard and Taylor (11) and Sanford (14). Weindling (18), Weindling and Fawcett (19), Christensen (3) and others have all shown that soil-inhabiting trichodermas suppress to a certain degree the virulence of plant pathogens found in the soil.

As opposed to cases cited where inhibition or antagonism between organisms has been exhibited with resultant lessening of the pathogenicity of the parasite, there are reports of stimulation. Thus, Sanford and Broadfoot (15) report that the filtrates of certain fungi and bacteria increased the severity of the injury from *O. graminis*. This phenomenon has been reported by Fawcett (4) and by others. Waksman (20) who has done a great deal of work concerned with components of the soil and activities of the soil flora has demonstrated that sterilized natural substrata are seldom, if ever, decomposed as completely by pure cultures as by a mixture of organisms from a natural soil.

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The experiments to be presented deal with inoculation studies concerning the two principal pathogenes causing common rootrot of cereals in Western Canada, *Helminthosporium sativum* and *Fusarium culmorum*. Preliminary experiments had indicated some form of antagonism between these two fungi when they were associated with one another on wheat seed. Hynes (9) reported studies in which *H. sativum*, *Helminthosporium* M, *Fusarium* sp. (of the *culmorum* type), and *O. graminis* were used to inoculate wheat seed singly and in various combinations. He found that *Fusarium* sp. intensified the activity of *H. sativum* when associated with it and reduced the severity of *Helminthosporium* M. In his experiments he used sterilized soil and for inoculation purposes he cultured the fungi on sterilized oats. These, together with differences in environmental and other conditions may well explain what might appear to be a discrepancy between his findings and those reported in the present paper.

GREENHOUSE STUDIES

Materials and Methods

Glazed gallon-crocks were used in all greenhouse tests. Unsterilized soil and sand in a 5 : 1 ratio were screened, thoroughly mixed, and an equal weight placed in each container. The mixture was adjusted to 50% of its moisture holding capacity. Thatcher wheat was employed throughout, seeded about one and one-half inches in depth. A greenhouse temperature of around 70° F. was maintained and emergence counts were taken 10 days after the experiments were planted. Each treatment was replicated four times.

Methods of inoculation were by means of a spore suspension and by the use of fresh cultures of the fungi on ground oat hulls. Where the former method was used a suspension of the conidia of each fungus was prepared from young cultures grown on potato-dextrose agar. Equal quantities of the suspensions were mixed together to give the combined suspension. A like amount of each of the single suspensions was saved and to each was added an equal volume of water. This procedure makes the number of spores of each fungus, per unit of suspension, the same in the single as in the combined suspensions. The method of application was to add a quantity of the suspension equivalent to one-tenth the weight of the seed used and to shake it up thoroughly (13). The seed was allowed to dry over night then kept in open packets for two days prior to seeding. The check samples received a distilled water treatment. In oat-hull inoculations where combinations of the two fungi occurred, the cultures were mixed together thoroughly and 12 grams was placed on top of the seed in each crock, as opposed to 6 grams in the single inoculations. A problem arose here in connection with the checks. In a preliminary test oat-hull cultures of *H. sativum* and *F. culmorum* were mixed in equal quantities, sterilized, then applied as in the inoculated crocks. In this case the emergence was very much reduced. The injurious effects may be due to toxic substances produced in the growth of the fungi or else the sterilized inoculum acts as a culture medium for fungi present in the soil (13). As a result it was considered inadvisable to use any treatment on the checks.

In the first experiments, *Ophiobolus graminis* Sacc. was included in addition to *H. sativum* and *F. culmorum*. Inoculation with *O. graminis* was by means of small discs of potato-dextrose agar overgrown with the fungous mycelium.

Experimental Results

As a preliminary test, wheat seed was inoculated with *H. sativum*, *F. culmorum* and *O. graminis*, singly and in all combinations. The spore-suspension method was used with the two former fungi and mycelial discs in the case of the latter. The data taken were confined to emergence counts, taken at 10 days and 20 days after planting. The later count was necessitated because killing of plants by *O. graminis* usually takes place several days after emergence. For the purposes of this test, plants killed in the period between the first and second counts were classified along with the non-emerged.

Analysis of the data showed that the treatments, *H. sativum*, *O. graminis* and the interaction *H. sativum* × *F. culmorum*, were significant beyond the 1% point. The two former treatments were each responsible for a decrease in emergence while the effect of the interaction *H. sativum* × *F. culmorum* was to increase the emergence. None of the other treatments produced statistically significant increases or decreases. *O. graminis* did not appear to influence the other fungi when associated with them nor did they affect its action on the seedlings. On the other hand, Hynes (9) reported that a weak strain of *O. graminis* reduced the amount of pre-emergence blighting due to *H. sativum*. A possible explanation of these differences may be in the fact the he used sterilized soil while the present study was conducted with unsterilized soil. In the report of Sanford and Broadfoot (15) neither *H. sativum* nor *F. culmorum* are classed as displaying any measure of control on *O. graminis*. Since the interaction *H. sativum* × *F. culmorum* suggested antagonism it was decided to investigate further this phase of the test.

In later greenhouse work, wheat seed was inoculated with *H. sativum* and *F. culmorum* singly and in combination, employing two methods of inoculation, spore suspension and oat hulls. Emergence and yield notes were taken. These are presented in Table 1 and their analyses in Table 2. The emergence notes were somewhat complicated in that the checks for the oat-hull treatment, which received an application of oat hulls overgrown

TABLE 1.—EMERGENCE AND YIELD DATA FROM A GREENHOUSE TEST

Treatment	Inoculation method	Emergence	Yield*
		No.	gm.
Check	Spore suspension	178	30.6
H. sativum	Spore suspension	120	38.5
F. culmorum	Spore suspension	103	33.7
H. sativum + *F. culmorum*	Spore suspension	106	31.4
Check	Oat hulls	178	35.4
H. sativum	Oat hulls	35	38.4
F. culmorum	Oat hulls	100	36.0
H. sativum + *F. culmorum*	Oat hulls	88	41.7

* Populations were equalized after emergence counts had been taken.

TABLE 2.—MAIN AND INTERACTION EFFECTS FROM DATA PRESENTED IN TABLE 1.

Treatment effect	Effect* on	
	Emergence	Yield
	No.	gm.
Check	—	—
H. sativum	**−210**	*13.0*
F. culmorum	**−114**	− 4.8
H. sativum × *F. culmorum*	**192**	− 6.2
Oat hulls	**−106**	**22.0**
H. sativum × oat hulls	**−100**	3.2
F. culmorum × oat hulls	**64**	1.8
H. sativum × *F. culmorum* × oat hulls	**70**	*14.2*

* Data in bold-face and italics exceed the 1% and 5% levels of significance respectively.

with the fungi and then sterilized, showed a very much reduced emergence. For purposes of analysis the emergence data for the checks of the spore suspension series were also used for the checks of the oat-hull series. It was found that a variation equal to the coefficient of variability (12%) in the substituted values did not materially alter the significance of the results.

It will be seen from Table 2 that *H. sativum* was responsible for a highly significant decrease in emergence. Moreover, the interaction *H. sativum* × oat hulls shows that the oat-hull method of inoculation caused a greater decrease than the spore suspension method. *F. culmorum* produced a highly significant decrease but in this case the spore suspension method was more effective in reducing the emergence. One might expect to get some cumulative effect under conditions of combined inoculation and that the emergence would be less than where either of the organisms was used singly. However, the data in Table 1 show that while the two fungi in combination reduced the emergence, they did not lower it as much as the more aggressive of the single inoculations. From the treatment effects which are given in Table 2, it may be seen that the interaction of the two fungi under either method of inoculation caused a highly significant increase in emergence. The increase was greater with the oat-hull method. In this connection the findings of Garrett (5) are of interest; he has reported that antagonism is much more pronounced with cultures of the fungus in organic media than with spore suspensions. After taking the emergence counts the population in each container was thinned down at random to a uniform number of plants consistent with the relatively small amount of soil in each crock. It is realized that this procedure does not entirely simulate field conditions and that weakened plants are given an undue advantage. It was found at harvest that inoculation with *H. sativum* caused a statistically significant increase in yield. While this may appear anomalous it is in line with the findings of Sallans (12). He found that after the initial set-back following infection, recovery occurred when moisture was adequate. This recovery was more than a mere catching up, in that the plants in later stages produced more and larger leaves with consequent increased photosynthetic areas, greater use of water and larger yields. Injury to the seedlings was presumably less where the two fungi

were mixed than where *H. sativum* was used alone. Keeping this in mind and also the effect of inoculation with *H. sativum* on yield, it might be deduced that the phenomena relevant to increased yield would not be operative where infection was reduced due to association of the fungi. The oat-hull method of inoculation caused a highly significant increase in yield over the spore-suspension method, also its effect on the interaction *H. sativum* × *F. culmorum* was a significant increase. Whether these increases were due wholly to intensified biological activity dependent on the additional organic matter or to fertilizer effects is unknown. Both factors may have been concerned.

FIELD EXPERIMENTS

Materials and Methods

In 1940 field tests were conducted at Saskatoon and Indian Head. Thatcher wheat was inoculated with *H. sativum* and *F. culmorum*, both singly and in combination. Two methods of inoculation were used, spore suspension and oat hull. The spore suspensions were made up and applied as described in the greenhouse tests above. The oat-hull media was applied at the rate of 20 grams per 18½-foot row. Where the two fungi were used in combination, 20 grams of each was put into the rows. The check rows for the spore-suspension group were treated with distilled water and the checks for the rows inoculated by the oat-hull method were left untreated. A Kemp V-belt seeder was used and the oat-hull inoculum was scattered in along with the seed. The test was laid out in properly randomized 5-row plots, replicated four times and the emergence was taken 18 days after seeding. At harvest time one foot was discarded from the ends of each plot and only the three centre rows were taken for yield data.

Experimental Results

As will be seen from Table 3, the responses to the various treatments were quite similar at both locations. The emergence, however, was lower at Saskatoon while the yields were somewhat higher at the same station. These differences can be explained on a basis of moisture and soil conditions. In Table 3 are given the emergence and yield notes obtained at Saskatoon and Indian Head and in Table 4 the analyses of these data using the method of Yates (21).

TABLE 3. EMERGENCE AND YIELD DATA OBTAINED IN FIELD TESTS CONDUCTED AT TWO LOCATIONS IN 1940, USING TWO METHODS OF INOCULATION

Treatment	Inoculation method	Saskatoon		Indian Head	
		Emergence	Yield	Emergence	Yield
		No.	gm.	No.	gm.
Check	Spore suspension	2055	3262	2222	2490
H. sativum	Spore suspension	1092	2931	2108	2506
F. culmorum	Spore suspension	405	1885	827	1930
H. sativum + *F. culmorum*	Spore suspension	369	1520	909	1996
Check	Oat hulls	2065	3107	2243	2530
H. sativum	Oat hulls	1053	2432	1098	2370
F. culmorum	Oat hulls	815	2411	1329	2414
H. sativum + *F. culmorum*	Oat hulls	1001	2644	1141	2238

[illegible]

[illegible]	[illegible]		[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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strip of agar about 5 mm. in width. This corresponds very well with type 1(A) as illustrated by Broadfoot (2). Only a slight degree of incompatibility was displayed between colonies of *F. culmorum*.

These tests indicate that on potato-dextrose agar, *H. sativum* is quite sensitive to other colonies either of itself or *F. culmorum*. *F. culmorum* on the other hand is not inhibited to the same degree. As Broadfoot (2) has shown, results obtained on one substrate may not be applicable when the fungi are grown on a different medium, therefore, one cannot apply the foregoing reactions to field conditions.

Germination Tests

Conidial suspensions of the two fungi in question were made up, singly and in combination, then transferred to the surface of a thin film of clear agar on a microscope slide. Thus on each slide there was *F. culmorum* alone, *H. sativum* alone, and also the two mixed together. The slides were placed in a petri-dish moist-chamber and after an incubation period of about 6 hours they were examined under a microscope and a count taken on the percentage of spores germinated.

In view of the smaller size of *F. culmorum* conidia, the suspensions were made up so that in combination there were always numerically more of these. The ratio varied from 3 to 1 to 20 to 1. In Table 5 are presented the results of all tests conducted. In each case the basis is on a spore count of 100.

Table 5.—Percentage germination of conidia of *H. sativum* and *F. culmorum* singly and when combined

Test	*H. sativum*	*F. culmorum*	Conidia mixed	
			H. sativum	*F. culmorum*
	%	%	%	%
1	87	24	25	98
2	25	95	5	90
3	90	70	12.5	Germination good
4	77	54	30	Germination good
5	71	Germination good	41	93
6	16	10	1	1
7	14	1	0	0
8	18	70	4	70
Total	398	324	118.5	352
Average	49.7%	46.3%	14.8%	58.7%

It will be seen from Table 5 that there was a good deal of variation in the percentage germination from test to test. There are a number of factors that probably contribute to cause this. First the cultures from which the conidia were taken varied in age from four days to ten days in the different tests. In any one test, however, the cultures used were the same age and grown on potato-dextrose agar from the same batch. The different tests were conducted at intervals, the last ones several months later than the first so that there may have been quite distinct differences in the media used. The same isolates of both fungi were used throughout.

In spite of these variations it is seen that the trend has been rather uniform throughout. In general, *H. sativum* germinated well when alone, the same was also true of *F. culmorum*. When the two were combined, however, the situation was different. The conidia of the latter fungus germinated on the average as well as when alone, but those of *H. sativum* germinated at a much reduced rate.

Further tests were conducted in which wheat kernels were used as a medium for conidia germination. They were washed thoroughly with distilled water, inoculated with the fungi under discussion and allowed to incubate in a moist chamber. Pieces of epidermis were stripped off and the spores examined under a microscope. With this method it was very difficult to make a satisfactory count when the suspensions were heavy. However, data taken indicated that the germination of *H. sativum* was reduced. When light suspensions of the spores were used satisfactory counts could be made, but there did not appear to be significant differences between the germination of *H. sativum* singly and in combination with *F. culmorum*. A heavy suspension was considered to be 12 to 20 spores of *H. sativum* and 60 to 100 of *F. culmorum* per high power field.

DISCUSSION

When *Helminthosporium sativum* and *Fusarium culmorum* were used in conjunction with one another on a seed sample, the injury as evidenced in emergence was lessened, pointing to possible antagonism between the two fungi. It was also found that antagonism was more marked where the oat-hull method of inoculation was used. It is likely that inoculation with oat hulls would allow more of the toxic substances to be carried into the zone of infection and these would retard the growth and parasitic activities of one or both fungi.

In the experiments conducted only a single isolate of each fungus was used. The results might have differed had other isolates been tested. Weindling (18) working with *Trichoderma lignorum* has shown that some strains are much more aggressive than others in the production of a lethal principle. Recently Sanford and Cormack (16) have reported differences in the ability of random isolates with a given species to antagonize *H. sativum*. This may explain why Hynes (9) got results different from those reported here even though he used the same species of fungi. It must be remembered, however, that he cultured the fungi on a different medium and that his experiments were conducted in sterilized soil.

Laboratory tests showed that the germination of conidia of *H. sativum* was hindered in the presence of *F. culmorum* and while it cannot be definitely deduced that this is what occurs under field conditions it is at least suggestive. Mead (10) found that treatment of seed with organic mercurial compounds would inhibit the germination of *H. sativum* on potato-dextrose agar but that if the conidia were germinated before the fungicide was applied the effect on development of the fungus was much less severe. *F. culmorum* as judged by its growth on potato-dextrose agar appears to be a fungus of the non-staling type, therefore, it is not probable that the inhibitory agent concerned is a staling product. According to Weindling (17) staling substances of fusaria and other fungi seem to be obtained

most abundantly from dead cells. While the same may be true for *F. culmorum* it is of interest that germination of the conidia of *H. sativum* was reduced through association with conidia from 4-day old cultures of *F. culmorum* on potato-dextrose agar.

In conclusion, it might be stated that great care should be used in interpreting the results of inoculation experiments with mixed cultures.

SUMMARY

It was shown in this work that *Helminthosporium sativum* and *Fusarium culmorum*, common rootrot fungi, would almost invariably cause in inoculation tests a reduction in the emergence and frequently in yield of wheat. This has been shown many times by previous workers. On the other hand, in inoculation tests where the two fungi were mixed, injury as reflected in emergence and sometimes in yield as well, was noticeably less, indicating antagonism. It was further shown that antagonism was more marked where the oat-hull method of inoculation was employed. In laboratory tests it was demonstrated that the germination of *H. sativum* conidia was reduced by the presence of conidia of *F. culmorum*.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. P. M. Simmonds for helpful suggestions and criticisms regarding this investigation and Dr. B. J. Sallans for assistance in the organization and analyses of greenhouse and field experiments.

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FURTHER DATA ON THE RELATION BETWEEN SHELL STRENGTH, POTENTIAL HATCHABILITY AND CHICK VIABILITY IN THE FOWL

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[Received for publication January 29, 1942]

Following the author's original report (1) on the relation of specific gravity of the egg to its potential hatching power based on experiments conducted at Ottawa further trials were carried out at a number of Branch Farms of the Dominion Experimental Farms Service in the 1940 and 1941 hatching seasons, and in addition a great deal more work was conducted at Ottawa. In the great majority of cases the original Ottawa results were fully confirmed. However, there were certain exceptions to which attention will be drawn in this paper and which make it clear that under certain conditions, variations between eggs in respect to their specific gravity (percentage shell) are not related to potential hatchability.

In the original work the specific gravity of each egg was determined to the nearest .004 place by determining their floating level in a series of salt solutions of known strength. As a result of that work it was found that Leghorn eggs have a higher average specific gravity than Barred Rocks eggs. A curvilinear relationship was found between specific gravity and hatchability, and for practical purposes it was decided that Barred Rock eggs which were less than 1.077 in specific gravity and Leghorn less than 1·085 could be profitably discarded for incubation purposes and replaced by heavier shelled eggs. These separation points, i.e. 1.077 and 1.085 are generally referred to as critical points.

NEW DATA

All the eggs set during March and April 1940 at eight branch farms were classified as either above or below the 1.077 point specific gravity. The stock kept on these farms varied as to breed, five farms having Barred Rocks, two White Wyandottes and one White Leghorns. Complete hatchability data were recorded. The data are summarized in Table 1 and it can be seen that, with the exception of the Wyandottes at Summerland, there is a consistent and appreciable difference in favour of the higher specific gravity eggs. The Summerland Wyandottes show a difference of 14% in total eggs hatching in favour of the weaker shelled eggs, but since only 2.6% or 18 of the eggs from this flock were below the critical point, the numbers were insufficient to establish a true difference.

The most unusual thing about the data in Table 1 is the very high proportion of floaters among the Kentville eggs. When this first became evident the hydrometer and the technique were carefully checked but nothing could be found which indicated that the results were spurious. The conclusion was reached that the Kentville eggs were actually much weaker in shell than the Leghorn eggs at Ottawa which represented the only other Leghorn flock from which data had been secured at that time. Nevertheless, the hatchability was exceedingly high and the usual sharp difference in the hatching power of the sinkers and floaters existed within the farm.

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On the whole, therefore, the 1940 work carried out on eight farms from coast to coast on a total of over 15,000 eggs confirmed the previous year's results which were obtained at Ottawa on a little more than 5,000 eggs (1).

During the hatching season of 1941 the work was continued on seven branch farms and the results secured are summarized in Table 2. In this table, the three farms at Charlottetown, Fredericton and Kentville have given unusual results. In the case of the first two the hatchability of the sinkers and floaters is about equal, while at Kentville there is a slight but insignificantly higher level of fertility and hatchability in the floaters. At Kentville the same very high proportion of floaters as occurred the previous year is again evident. A second Leghorn farm at Saanichton is included in the 1941 results and shows a higher proportion of floaters than the Ottawa Leghorn eggs but not nearly as high as the Kentville eggs. No explanation for the low percentage of shell on the Kentville eggs is yet apparent. It would not appear to be a matter of strain because six adult Kentville females which were moved to Ottawa in December 1938 produced eggs which were equal in specific gravity to the eggs from Ottawa birds during the 1939 hatching season and higher than those from their full and half sisters retained at Kentville. Subsequent exchanges of stock between the two farms have confirmed the view that environmental differences between the farms is responsible for the difference in shell strength.

At Lethbridge, only 4 out of 2,758 eggs (or 0.1%) fell below the 1.077 point and thus the eggs at Lethbridge must have been much stronger shelled than those from most Barred Rock farms. In the previous year, however, 23% of the eggs produced at Lethbridge fell below 1.077; here again it is obviously an environmental difference between years which has been responsible for the variation.

Specific Gravity of the Egg and Chick Viability

In the previous paper (1) some evidence was presented which strongly indicated that chicks which hatched from low specific gravity eggs were weaker and had a higher proportion of deaths in early life than those from stronger shelled eggs. However, the data were insufficient to prove the point. Additional data from a number of branch farms regarding this point are summarized in Table 4.

Of the six flocks in which comparisons can be made all but one show an appreciably higher death rate in chicks hatched from the weaker shelled eggs. When these paired mortality rates are analysed by Student's method a t value is obtained which equals odds of 50 : 1 on significance. We may safely conclude, therefore, that not only do low specific gravity eggs give few chicks but also that a significantly greater proportion of these chicks die during early life than those hatched from high specific gravity eggs.

DISCUSSION

It is clear from the new data summarized in this paper that there is considerable variability in the percentage of shell in eggs from different flocks as well as in eggs from the same flock at different times. Furthermore the relationship between percentage shell and hatching power is not constant, and in some cases the circumstances are such that eggs below the critical point hatch equally as well as those above.

Factors affecting shell formation are undoubtedly numerous and complex. The fact that in most cases a very definite relationship exists between shell strength and both fertility and hatchability means that some of these factors also affect the embryo, presumably by way of the chemical makeup of the egg. However, other and what may be termed residual factors exist which also affect the shell but are not related to the embryo. If these theoretical arguments are correct, and presuming the residual factors to be more or less constantly present in all flocks, it would appear that populations of eggs in which no relationship between shell strength and specific gravity exists have presumably been subject only to factors causing residual variability, and they should be characterized by a lesser degree of variability about the mean with respect to shell strength than populations showing a marked degree of relationship between specific gravity and hatchability, these latter being acted upon by factors responsible for the relationship as well as by the residual factors. Existing data for testing this hypothesis are not extensive since measures of variability can only be secured in cases where the specific gravity of each egg was determined. In the case of Barred Rock eggs such information is available only at Ottawa and here specific gravity and hatchability have always been quite closely related. However, the required information does exist for the Kentville White Leghorn eggs which showed no relationship between specific gravity and hatchability, and for comparison there is similar information from White Leghorn eggs at Ottawa and Saanichton, both of which showed a correlation between specific gravity and hatchability. Table 3 summarizes these data and it can be seen that at Kentville the variance was considerably less than at Saanichton where a fair relationship existed between specific gravity and hatchability, and very much less than at Ottawa where this relationship was strong. While more egg populations, particularly those showing no relationship between specific gravity and hatchability, would be necessary before the point could be definitely proved, the numbers in each of the groups in Table 3 are adequate to provide accurate estimates of the variance of an infinite population under similar conditions and the evidence thus lends strong support to the theory. Incidentally, Table 3 shows quite definitely that the average shell strength as measured by specific gravity is no indication of whether the relationship between specific gravity and hatchability exists. In the beginning it was thought that those farms with low hatchability would have a low specific gravity and there would be, therefore, correspondingly greater opportunities for improving hatchability through selection of eggs by the specific gravity test. This has not proved to be the case as is readily seen in Table 3. Kentville eggs have a hatchability rate approximately equal to those both at Saanichton and Ottawa, and still these three farms are widely divergent in degree of variability and in relationship between shell and hatchability. Two groups of eggs collected at different seasons are shown for Ottawa, and although these are from the same flock of hens the second lot is much lower than the first in shell strength. This again emphasizes the importance of environment in determining shell strength. Although the specific gravity in this second Ottawa lot is almost as low as that at Kentville, the variance and specific gravity—hatchability relationship are at the opposite extreme. It seems, therefore,

that the actual level of specific gravity for a group of eggs is little or no indication of their hatching power as compared with a second group from a different source and that neither the level of specific gravity or of hatchability will indicate whether there exists a relationship between them. However, it would seem that the existence of this relationship might be indicated by the level of the variance. While differences between flocks with respect to specific gravity of the eggs does not accurately indicate the level of hatchability, data presented in this and the preceding paper show that differences in specific gravity between hens within flocks or between eggs within hens within flocks or more generally between eggs within flocks does in most cases indicate hatching power.

The important point to be noted is that a simple physical test of the new laid egg (percentage shell) is a measure of a complex physiologically controlled characteristic, hatchability. Since even in very weak shelled eggs there is more than sufficient calcium to supply the needs of the embryo, it would appear that the factors affecting hatchability do not lie within the shell itself. It seems more logical to regard the shell as a mirror of internal egg quality factors affecting hatchability. This places a new tool in the hands of researchers concerned with hatchability.

SUMMARY

While in the great majority of cases variability between new-laid eggs with respect to their specific gravity or percentage of shell is closely related to potential hatchability, there are exceptions to this rule. Under certain conditions the variability in this respect produced by a flock of birds bears no relationship to hatching power.

It is suggested that certain relatively constant residual variables exist which affect calcium metabolism and thus the egg shell, quite independently of the egg contents. In addition there generally exists a second group of variables which affect both egg shell and egg contents and therefore the ability of the egg to become fertilized and support the embryo. Where only the first or residual type of variability exists the egg shells are quite uniform and show no relation to hatchability, but where both types operate the variability is increased and is related to hatching power.

The exact nature of these variables is unknown. They are undoubtedly physiological or at least produce their effect through physiological mediation.

Differences between flocks with respect to the average specific gravity of the eggs produced do not seem to be reliable indices of the hatchability of the flocks, but differences between the average of hens within flocks or between individual eggs within flocks or within hens are generally related to hatching power.

Chicks hatched from low specific gravity eggs have a higher death rate in early life than those from high specific gravity eggs.

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TABLE 1.—RESULTS OBTAINED ON BRANCH FARMS IN THE HATCHING SEASON (MARCH AND APRIL) 1940. SINKERS ARE EGGS WHICH ARE HIGHER IN SPECIFIC GRAVITY THAN THE CRITICAL POINT, WHILE FLOATERS ARE LOWER

Location	Eggs set		No. fertile	No. hatch	Percentage fertile	Percentage fertile hatch	Percentage total hatch	Percentage floaters
	Barred Rocks—critical point 1.077							
Brandon, Man.	Sinkers	766	703	592	91.8	84.2	77.3	
	Floaters	757	678	443	89.6	65.3	58.5	20.0
	Total	1523	1381	1035	90.7	74.9	68.0	
	Barred Rocks—critical point 1.077							
Charlottetown, P.E.I.	Sinkers	1489	1166	924	78.3	79.2	62.1	
	Floaters	245	189	139	77.1	73.5	56.7	14.1
	Total	1734	1355	1063	78.1	78.5	61.3	
	Barred Rocks—critical point 1.077							
Fredericton, N.B.	Sinkers	2941	2667	1789	90.7	67.1	60.8	
	Floaters	1440	1235	716	85.8	58.0	49.7	32.9
	Total	4381	3902	2505	89.1	64.2	57.2	
	Barred Rocks—critical point 1.077							
Lethbridge, Alta.	Sinkers	1729	1514	1160	87.6	76.6	69.1	
	Floaters	516	430	246	83.3	57.2	47.7	23.0
	Total	2245	1944	1406	86.6	72.3	62.6	
	Barred Rocks—critical point 1.077							
Nappan, N.S.	Sinkers	2651	2422	1791	91.4	73.9	67.6	
	Floaters	190	140	82	73.7	58.6	43.2	14.7
	Total	2841	2562	1873	90.2	73.1	65.9	
	White Wyandottes—critical point 1.077							
Lacombe, Alta.	Sinkers	1126	1018	625	90.4	61.4	55.6	
	Floaters	96	91	44	94.8	48.4	45.8	7.9
	Total	1222	1109	669	90.8	60.3	54.7	
	White Wyandottes—critical point 1.077							
Summerland, B.C.	Sinkers	662	594	530	89.7	89.2	80.1	
	Floaters	18	18	17	100.0	94.4	94.4	2.6
	Total	680	612	547	90.0	80.4	80.4	
	White Leghorns—critical point 1.085							
Kentville, N.S.	Sinkers	74	71	55	95.9	77.5	74.3	
	Floaters	413	384	244	93.0	63.5	59.1	84.8
	Total	487	455	299	93.4	65.7	61.4	

TABLE 2.—RESULTS OBTAINED ON BRANCH FARMS IN THE HATCHING SEASON OF 1941

Location	Eggs set		No. fertile	No. hatch	Percentage fertile	Percentage fertile hatch	Percentage total hatch	Percentage floaters
	Barred Rocks—critical point 1.077							
Nappan, N.S.	Sinkers	886	757	521	85.4	68.8	58.8	
	Floaters	124	80	42	64.5	52.5	33.9	12.3
	Total	1010	837	563	82.9	67.3	55.7	
	Barred Rocks—critical point 1.077							
Lethbridge, Alta.	Sinkers	2758	2608	1999	94.6	76.6	72.5	
	Floaters	4	4	2	100.0	50.0	50.0	0.1
	Total	2762	2612	2001	94.6	76.6	72.4	
	Barred Rocks—critical point 1.077							
Brandon, Man.	Sinkers	4324	3773	2799	87.3	74.2	64.7	
	Floaters	1068	820	568	76.7	69.3	53.1	19.8
	Total	5392	4593	3367	85.2	73.3	62.4	
	Barred Rocks—critical point 1.077							
Charlottetown, P.E.I.	Sinkers	985	731	539	74.2	73.7	54.7	
	Floaters	401	310	223	77.3	71.9	55.6	28.9
	Total	1386	1041	762	75.1	72.3	55.0	
	Barred Rocks—critical point 1.077							
Fredericton, N.B.	Sinkers	2018	1886	1174	93.5	62.2	58.2	
	Floaters	429	404	253	94.2	62.6	59.0	17.5
	Total	2447	2290	1427	93.6	62.3	58.3	
	White Leghorns—critical point 1.085							
Kentville, N.S.	Sinkers	1071	1019	801	95.1	78.6	74.8	
	Floaters	1653	1555	1273	94.1	81.9	77.0	60.7
	Total	2724	2574	2074	94.5	80.6	76.1	
	White Leghorns—critical point 1.085							
Saanichton, B.C.	Sinkers	578	533	454	92.2	85.2	78.5	
	Floaters	297	256	196	86.5	76.5	66.0	33.9
	Total	875	789	650	90.2	82.4	74.3	

TABLE 3.—COMPARISON OF THE DEGREE OF VARIABILITY IN SPECIFIC GRAVITY (VARIANCE) BETWEEN GROUPS OF EGGS SHOWING VARYING DEGREE OF RELATIONSHIP BETWEEN SPECIFIC GRAVITY AND HATCHABILITY

Farm	No. eggs	Date laid	Average specific gravity	Variance	Relationship S. gravity and hatchability
Kentville	2724	March and April 1941	1.0830	17.76	None
Saanichton	875	March and April 1941	1.0857	25.67	Fair
Ottawa	2549	March and April 1939	1.0894	29.47	Strong
Ottawa	138	September 1939	1.0843	49.26	Strong

TABLE 4.—COMPARISON OF MORTALITY IN CHICKS HATCHED FROM STRONG SHELLED (SINKERS) AND WEAK SHELLED EGGS (FLOATERS)

Farm	Breed	No. chicks from		Percentage chick mortality to 3 weeks	
		Sinkers	Floaters	Sinkers	Floaters
Charlottetown	B.P.R.	924	139	2.49	2.88
Lethbridge........	B.P.R.	1160	246	2.07	4.88
Nappan	B.P.R.	468	29	1.50	3.45
Ottawa	B.P.R.	1106	196	2.53	4.08
Ottawa	W.L.	1351	149	2.37	4.70
Summerland	W.W.	530	17	0.91	0
Total		5539	776	2.08	4.12

STUDIES ON THE THERMAL RESISTANCE OF HONEY YEASTS[1]

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[Received for publication November 20, 1941]

Since Barker instituted the generic name Zygosaccharomyces in 1901, more than forty species of these yeasts have been isolated from fermented honey, maple syrup, chocolate creams, fruit syrups, soja, and other sources. However, there have been few investigations of the heat resistance of this genus.

THERMAL DEATH POINT OF SUGAR TOLERANT YEASTS

Nussbaumer (29) inoculated flasks of equal parts of honey and sterile water with yeasts isolated from honey (Stamm B fermenting glucose, fructose, mannose, sucrose, dextrin, and galactose). The flasks were heated to 70° C. (158° F.) in a water bath, in duplicate, for periods of ½, ¾, 1, 1½ and 2 hours, reckoning from the time the contents reached 70° C. Checks showed fermentation in 4 days. One of the ½-hour flasks fermented. The conclusion reached was that heating at 70° C. for a full half-hour was necessary to kill the honey yeasts and their spores, also to sterilize a fermenting honey.

Marvin (25, 26) isolated five types of yeast-like organisms from samples of fermenting Wisconsin honey, and Marvin, Peterson, Fred, and Wilson (27) conclude that it is possible to prevent fermentation of honey by holding it at 37.8° C. (100° F.) for several months, or at 50° C. (122° F.) for 24 hours. They state also that if honey is heated very quickly to 71.1° C. (160° F.), poured into containers, sealed while hot and cooled soon afterwards, the colour and flavour will not be changed to any great extent and that fermentation is immediately and completely stopped.

Fabian and Quinet (15) isolated 25 yeasts from honey, which they placed in five groups, (I to V), classifying four groups as Zygosaccharomyces and the fifth as Torula. They investigated the thermal death points of these yeasts by inoculating honey and broth with heavy suspensions of cells and subjecting the cultures to temperatures ranging from 45° to 60° C. (113° to 140° F.). Spores were heated to a range of 55° to 75° C. (131° to 167° F.). Their recovery procedure was to dilute the honey 50% with broth, and the broth 50% with honey and incubate for three weeks. It required longer heating to kill the vegetative cells and spores in the honey, indicating a protective action in the medium of higher concentration. The temperature and length of time required to kill the vegetative cells appear in Table 1, with the results obtained by other workers.

Some of the same organisms were found in maple syrup by Fabian and Hall (13). The thermal death point of 25 yeasts, including these and other isolates, was determined by much the same technique, substituting maple syrup for honey. The authors' conclusions are presented in Table 1.

[1] Contribution from the Bee Division, Experimental Farms Service, Department of Agriculture, Ottawa, Canada. Abridgment of Part I of a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture, University of Toronto.
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A more comprehensive report on the time and temperature relations in destroying yeasts in honey is presented by Townsend (33). The author used a mixture of four species of Zygosaccharomyces and one Torula in honey. The cells were first grown in 80% honey broth, at 32° C. (89.6° F.), for six days and then stored in a refrigerator at about 0° C. (32° F.). Honey of 18.6% moisture was inoculated with the five broth cultures and tests were made with 3 ml. in a test tube of 100 × 12 mm. Since the yeast colonies from 1 ml. of inoculated honey were too numerous to be counted in one-millionth dilution, the concentration was probably greater than one billion cells per ml. A thermometer in the test tube reduced the thickness of honey to 3.5 mm. After heating, the samples remained at room temperature for a day before sub-culturing. Sixty per cent honey agar was employed for plating, using 50 : 50 honey dilution blanks. It is evident from Table 1 that it required more than twice the length of time for Townsend to obtain sterility as it did for Fabian and co-workers.

Table 1.—Temperature and time required for killing vegetative cells of sugar tolerant yeasts, as reported by different authors

Yeast	Medium	Temperature	Time	Authors
Zygosaccharomyces	Honey	70° C. (158° F.)	30 min.	Nussbaumer (29)
Zygosaccharomyces and Torula	Honey	60° C. (140° F.)	10 min.	Fabian and Quinet (15)
Zygosaccharomyces	Honey	71.1° C. (160° F.)	Momentarily	Marvin *et al.* (27)
Zygosaccharomyces	Honey	37.8° C. (100° F.)	Several months	Marvin *et al.* (27)
Zygosaccharomyces	Honey	50.0° C. (122° F.)	24 hrs.	Marvin *et al.* (27)
Zygosaccharomyces and Saccharomyces	Maple syrup	60° C. (140° F.)	10 min.	Fabian and Hall (13)
Zygosaccharomyces and Torula	Honey	60° C. (140° F.)	20+ min.	Townsend (33)

REVIEW OF LITERATURE

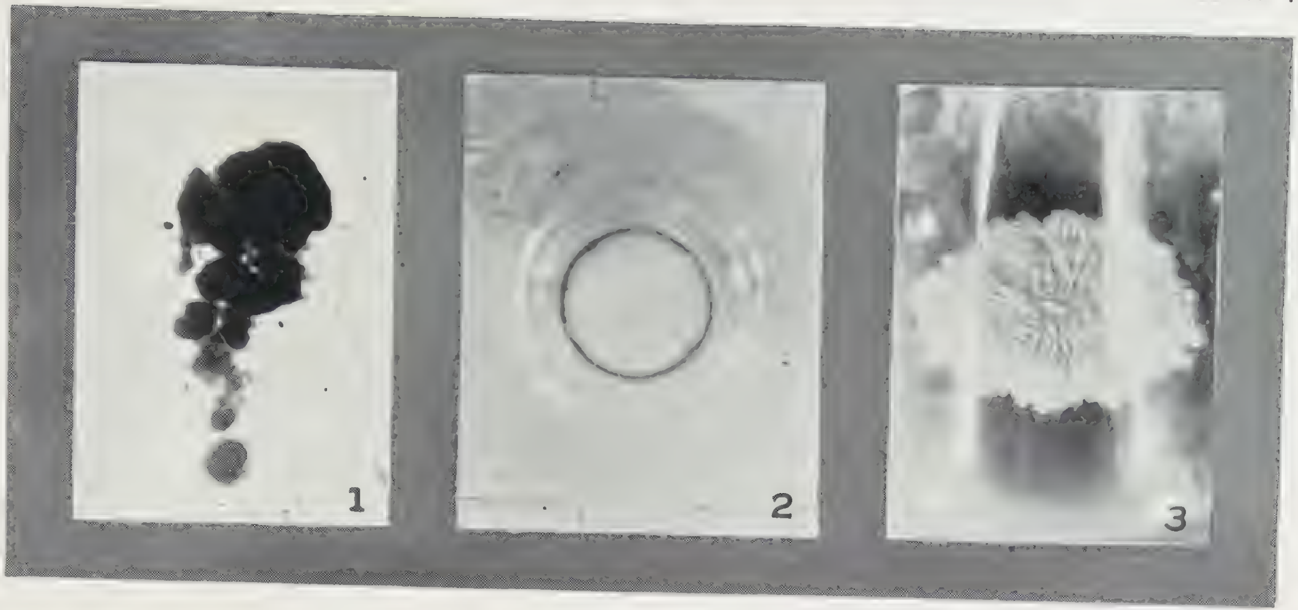
Beamer and Tanner (4) state that whatever is done to study the heat resistance of any group of micro-organisms must be with methods which have been developed largely with bacteria. Consequently the literature dealing with the heat resistance of bacteria has been reviewed in detail and some of the more pertinent factors are presented.

Methods of Determining Heat Resistance

The object of much of the work in recent years has been to reduce the number of "skips" which give misleading results, especially in qualitative tests. Although there may be great destruction in the number of cells, a few remaining cells cause fermentation. This fact led Robertson (31) to state that only data where quantitative determinations of the numbers surviving are accurately made are of real value and even these should be qualified with the conditions under which the tests are made.

Beamer and Tanner (3) have presented a detailed account of the methods used in determining the resistance of micro-organisms to heat. It is apparent that results obtained when using thin-walled capillary tubes, Sternberg bulbs, and Woulff bottles have not been entirely satisfactory.

Plate I



Single tubes of special glass sealed to contain aliquot portions have been used extensively but "skips" recurred constantly, and the use of duplicate and triplicate tubes made results more confusing.

Esty and Williams (12) compared single tube results with those obtained by using 25 to 30 tubes heated alike at given times, and Esty (10) after further investigation says that for all practical purposes this method should give reliable and fairly consistent information. He suggests, however, that for the establishment of standard type curves the percentage survival be determined for at least 4 well selected times on a large number of tubes (100 to 300 each time). Baker and McClung (2) have tested carefully open tube methods, concluding that it is necessary to use not less than 5 tubes, heating them for long periods at closely spaced heating temperatures.

Thermal Death Terminology

At present the generally accepted definition for the thermal death point of an organism is the lowest temperature which will kill it in 10 minutes. Buchanan and Fulmer (6) in discussing the use of this term state that in general it is probably best to discard it. Absolute and majority thermal death points have been determined on some organisms, but the more intensive research of Bigelow and Esty (5), Esty and Myer (11), Esty and Williams (12), Hastings, Fred and Carroll (16), Magoon (23, 24), Knaysi (17), Knaysi and Gordon (18), Rahn and Barnes (30), Aref and Cruess (1), Baker and McClung (2), and Beamer and Tanner (3, 4) shows that the conception of thermal death determinations must be comprehensive.

The term which is applicable in industry is "commercial sterility". This term, embodying the effect of majority killing, obviates the necessity of inclusive details and is more realistic than sterility, since it is recognized that a few viable organisms may survive even the most severe heating tests.

The Effect of Numbers of Cells

Esty (9), in extensive experiments with canned foods, has demonstrated that the greater the contamination, the heavier are the losses from under-sterilization, and results of most investigators confirm the declaration of Lang and Dean (19) that the magnitude of the resistance of spores to heat is in direct proportion to their concentration, provided the various concentrations are prepared from the same suspension of spores.

Strain, Age, and Concentration or Clumping of Cells

The factors inherent in the cells which affect their resistance to heat are strain, age, and the way in which they adhere in groups or clumps.

By continued culturing of resistant cells Williams (34) found that it was possible to increase the heat resistance of *Bacillus subtilis*, and Magoon (23) concludes that different strains of the same species often vary widely in their resistance to heat. This is also brought out in the work of Dickson, Burke, Beck, Johnston and King (8) who found the survival time of *Clostridium botulinum* heated at 100° C. (212° F.) to vary from 30 minutes to 6 hours.

It is usually considered that spores and older cells are more resistant than those whose physiological processes are functioning. Resting cells which are undergoing little physiological change are considered by Buchanan

Procedure

Uniform test tubes, 150 mm. by 14 mm. inside diameter, made of glass 1 mm. thick were filled with 14 gm. inoculated honey adjusted to contain 18% of water. The tubes were supported well apart in wire baskets and when immersed in the water bath the honey was one inch below the surface of the water. A thermometer was centrally adjusted in one of the tubes.

At the end of the required heating period the tubes were quickly transferred to ice water, and after cooling were enriched with 5 ml. nutrient solution. After thorough stirring 1.5 ml. amounts were taken directly, or in serial dilution, for recovery procedure.

Plates were poured with 30% honey agar of pH 4.8 for quantitative calculations. Qualitative results were obtained by inoculating tubes containing 9 ml. 2 : 1 honey broth of pH 3.8 with amounts equal to that used for plating. All were incubated at 30° C. (86° F.) and were held for 6 weeks if no evidence of growth or fermentation was present prior to that time.

Heating

I. Quintuplicate tubes were heated according to the recommendation of Baker and McClung (2) in a water-bath kept constant to ±0.5° C. (0.9° F.). In *(a)* and *(b)* a supplementary bath heated to 68° C. (154.4° F.) was used to shorten the pre-heating time before transferring to the constant temperature bath. In *(c)* the temperature of the bath was 90° C. (194° F.), transfer being made before the thermometer reached the required temperature. The particulars appear in Table 2.

II. Duplicate tubes were heated in a water-bath at 90° C. (194° F.). Because of the length of time required for the heat to penetrate the honey the thermometer registered the required temperature after transfer to ice-water, as is indicated in Table 2.

TABLE 2.—TEMPERATURES, TIMES, AND OTHER FACTORS CONCERNED WITH THE HEATING OF TUBES OF HONEY INOCULATED WITH SUGAR TOLERANT YEASTS

Expt.	Temp. to which tubes heated	Length of heating time	Pre-heating period	Temp. at time of transfer
I *(a)*	50° C. (122° F.)	5, 15, 30, 60 min.	1 min. 45″	45° C. (113° F.)
(b)	60° C. (140° F.)	5, 10, 20, 40 min.	2 min. 45″	58° C. (136.4° F.)
(c)	65° C. (149° F.)	Momentarily*	1 min. 50″	61° C. (141.8° F.)
	70° C. (158° F.)	Momentarily*	2 min. 10″	67° C. (152.6° F.)
	75° C. (167° F.)	Momentarily*	2 min. 35″	72° C. (161.6° F.)
	80° C. (176° F.)	Momentarily*	3 min. 10″	78° C. (172.4° F.)
II	75° C. (167° F.)	Momentarily*	2 min. 35″	72° C. (161.6° F.)

* Only one bath was necessary for the momentary heating tests. It was kept constant at 90° C. (194° F.).

Preliminary Investigations

Culture plates were poured with honey agar containing 15, 30, 45, 60, and 75% honey to determine the most satisfactory concentration. Thirty per cent honey agar was found to give rapid growth of yeasts so that reliable counts could be obtained on the fifth day, incubating at 30° C. (86° F.).

Tests were conducted to determine the effect of shaking the yeast cultures with glass beads for varying lengths of time. Microscopic examination revealed the presence of clumps, or chains of yeast cells, and their existence was evident in the appearance of orientated colonies which frequently appeared in plate cultures. Plate I, 1, shows a photomicrograph of one of these colonies. The time of shaking in the mechanical shaker was finally standardized at 30 minutes, although aggregates of cells were still apparent.

Main Investigations

I. The four cultures M1, J7, 138, and 139 were heated
   (a) at 50° C. (122° F.) for 5, 15, 30, and 60 minutes,
   (b) at 60° C. (140° F.) for 5, 10, 20, and 40 minutes,
   (c) to 65°, 70°, 75°, and 80° C. (149°, 158°, 167°, and 176° F.) momentarily.

II. The four cultures M1, J7, 138, and 139, were heated along with sixteen other yeast cultures at 75° C. (167° F.) momentarily.

RESULTS

I. The quantitative results obtained by heating the four Zygosaccharomyces species at 50° C. (122° F.) and 60° C. (140° F.) for varying lengths of time and the results of heating the yeasts momentarily at temperatures of 65°, 70°, 75°, and 80° C. (149°, 158°, 167°, and 176° F.) are given in Table 3.

Table 3.—Means of replicates of survivors of Zygosaccharomyces species heated at given temperatures for varying lengths of time

Temperature	Time	Species			
		M1	J7	138	139
	Check	121,000	153,000	2,672,000	126,200
	5 min.	57,150	57,350	840,900	68,820
50° C. (122° F.)	15 min.	28,320	37,240	849,900	4,365
	30 min.	6,493	9,410	232,030	2,932
	60 min.	478.2	3,737	150,570	340
	Check	126,900	159,400	2,288,500	115,600
	5 min.	0	3,412	1,525	0
60° C. (140° F.)	10 min.	0	429.1	257.7	0
	20 min.	0	5.8	29.5	0
	40 min.	0	0.3	1.6	0
	Check	43,800	186,100	324,900	73,000
65° C. (149° F.)	Momentarily	1,680	7,330	22,050	4,994
70° C. (158° F.)	Momentarily	119	103	8,245	998.5
75° C. (167° F.)	Momentarily	0	0	161.9	1.2
80° C. (176° F.)	Momentarily	0	0	1.4	0

Qualitative tests confirmed the results of the corresponding quantitative analysis in most of the cases. Occasionally the presence of very small numbers of yeasts would be unconfirmed by tubes failing to develop fermentation. More frequently the original tubes would ferment after inoculation and incubation, an occurrence which might be anticipated because of the increased amount of inoculum which they contained.

II. In Table 4 are shown the results obtained when inocula of 100,000 to 200,000 yeasts per ml. were heated in honey to 75° C. (167° F.) and immediately cooled. It is obvious that some yeasts are not as easily killed as others, and that resistant cells may give rise to strains of high resistance. This is borne out by 40-1 and 40–5 sub-cultures of J7 and M1, respectively, that had previously withstood a temperature of 60° C. (140° F.) for 40 minutes.

TABLE 4.—SURVIVORS OF CULTURES OF YEASTS ISOLATED FROM HONEY AFTER HEATING TO 75° C. (167° F.)

Culture	Plate count	Tubes	Fermentation
z.b.	3,500	++	Immediate
E6	2	++	Immediate
D1	0	+−	Delayed
J7	30	++	Immediate
M1	5	++	Immediate
N4	2	++	Delayed
N24	24	++	Delayed
138	0	++	Delayed
139	0	−−	None
40–60	20,000	−−	None
A2	12,000	++	Delayed
40–1	2,000	++	Immediate
40–2	0	++	Delayed
40–5	2,000	++	Delayed
H3	0	++	Delayed
H5	400	++	Immediate
BK5	18,000	++	Immediate
BK6	3,000	++	Immediate
BK7	8	++	Immediate
BK8	4	++	Immediate

"Immediate" fermentation indicates positive fermentation at the end of 1 week.

"Delayed" fermentation indicates tubes which showed no indication of fermentation at 1 week, but showed definite signs after 4 weeks' time.

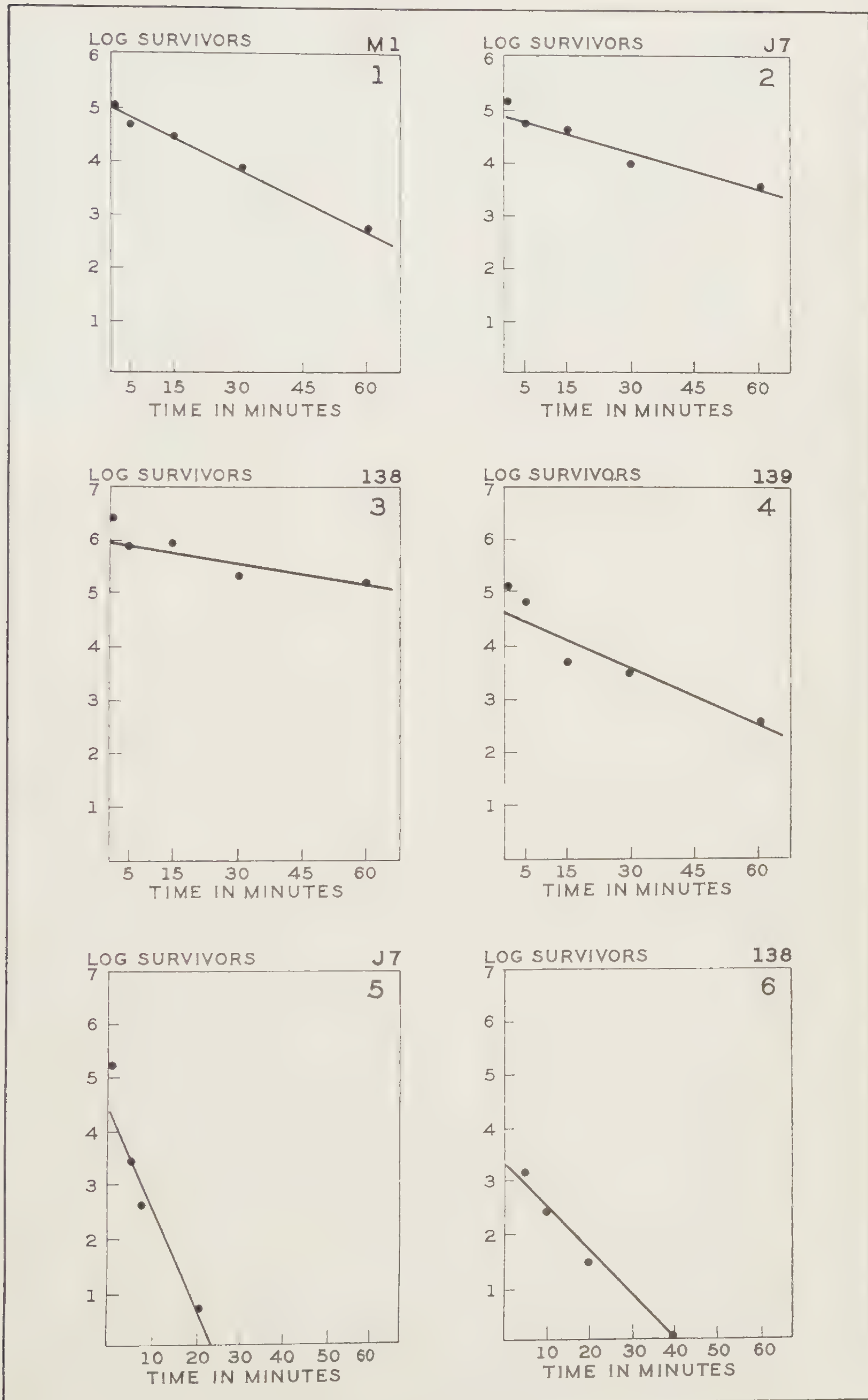
The signs, + and −, appearing in the third column indicate the presence, or absence, respectively, of fermentation in the original tubes.

DISCUSSION

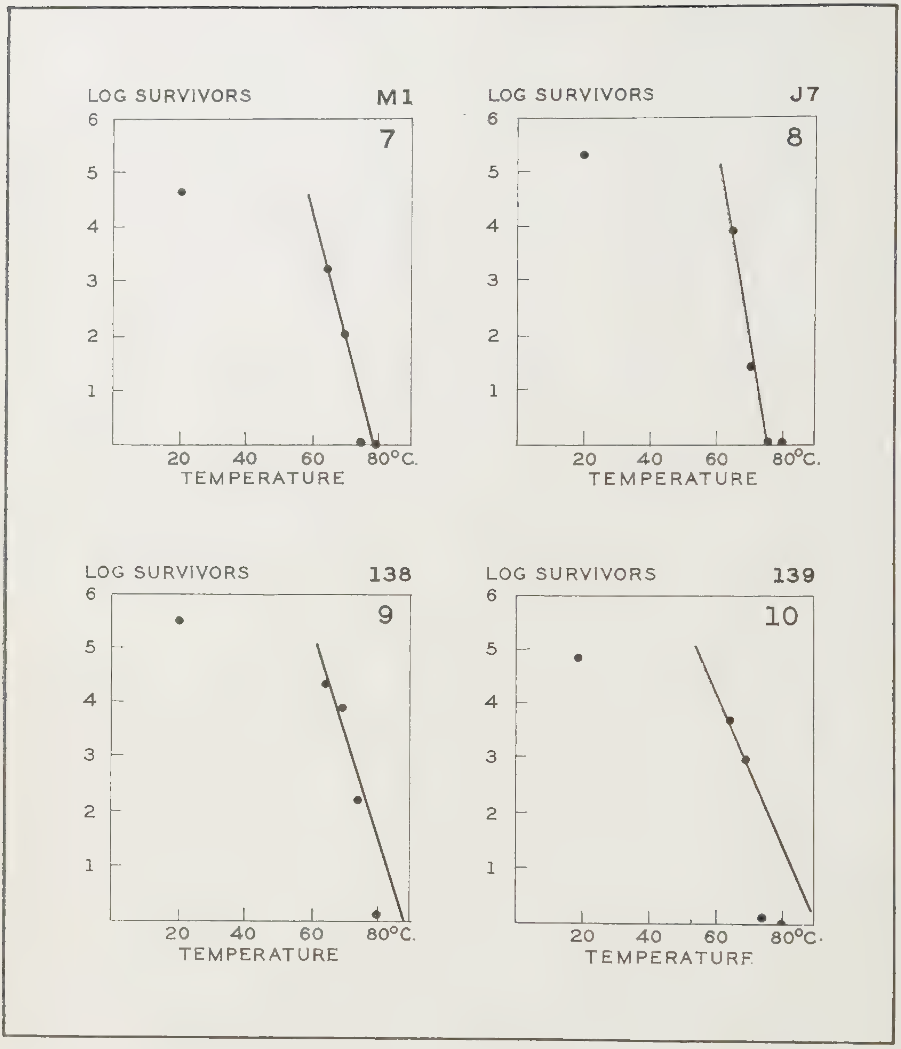
I. The quantitative results presented in Table 3 have been plotted on semi-logarithmic paper and are presented in Figures 1 to 10. The logarithm of survivors has been plotted against the length of time in minutes in Figures 1 to 6, but in the remaining figures (7 to 10), temperature replaces the length of time on the abscissa.

Examination of Figures 1 to 6 reveals that the plotted points do not lie far from the superimposed lines. There is a tendency towards concavity, especially in the case of those heated at 60° C. (140° F.). The extreme points were not included, however, in determining the line of best fit and in general the points in each figure may be considered as variants of an exponential equation.

Figure 3 brings out the fact that when 138 was heated 5 min. at 50° C. (122° F.) the killing was equally as great as at the 15 min. period (see Table 3). In this connection, it may be observed that whenever rapid killing occurred during a heating period, in the succeeding period the rate of death was decreased. Resistance in any one period has an apparent inverse ratio to rate of killing in the succeeding period. Also when this



FIGURES 1 TO 6.



FIGURES 7 TO 10.

effect is most pronounced in the first period the resulting decrease in rate of death would apparently be carried into succeeding periods. Thus Figures 2 and 3 show greater velocity than Figures 1 and 4 in the first 5-min. period but give final results considerably lower, as can be seen further in Table 5.

The effect of clumping of the cells must not be omitted here, as their presence was confirmed by microscopic examination and was evident in plate culture. (See Plate I, 1). Cells on the periphery of the cluster undoubtedly formed a protective coating for those nearer the centre and thus prolonged the heating period. The tendency of the different species to adhere closely would also be a factor in rate of death.

Velocity and Thermal Coefficients

It is obvious that these species of Zygosaccharomyces differed according to rate of dying, and in order that these rates may be compared the formula $k = 1/t \log B/b$ has been used to give numerical values.

k = velocity coefficient of rate of death
t = heating time
B = original count
b = survivor count

Values for the species which gave comparable results have also been compared as to rate of death at 60° C. (140° F.) with that at 50° C. (122° F.) and is recorded as $Q_{10}°$. These appear in Table 5, along with the original count, as calculated from the straight line relationship established in Figures 1 to 6.

TABLE 5.—THERMAL AND VELOCITY COEFFICIENTS FOR ZYGOSACCHAROMYCES SPECIES HEATED AT TWO DIFFERENT TEMPERATURES

Species	50° C. (122° F.)		60° C. (140° F.)		
	B	k	B	k	$Q_{10}°$
M1	121,000	0.038			
J7	153,000	0.022	159,400	0.185	8.41
138	2,672,000	0.015	2,288,500	0.111	7.40
139	126,200	0.037			

Species M1 and 139 appear to affect approximately the same resistance to the effect of heat at the lower temperature and to be more easily killed than J7 and 138, an assumption which is substantiated by their failure to survive a 5-min. heating period at the higher temperature. The lower k values of species J7 and 138 at 50° C. (122° F.) are followed by much higher ones at 60° C. (140° F.) at ratios indicated by the $Q_{10}°$ values.

While 138 has a lower thermal coefficient than J7 it had a very much larger original count, so much so that a still smaller $Q_{10}°$ value might be expected if the two species possessed the same thermal resistance. When heated at 75° C. (167° F.) I, species 138 had an indicative count, but when heated to this temperature in II there was no quantitative evidence of viability, although the original tubes gave delayed fermentation. In the comparative test J7 gave an indicative count and immediate fermentation.

Analysis of Variance

The quantitative results of heating at 50° C. (122° F.) and at 60° C (140° F.) for various lengths of time and those of heating to 65° and 70° C. (149° and 158° F.) momentarily appearing in Table 3, have been analysed by the variance method and the results are shown in Table 6, based on logarithms of the original numbers. Qualitative tests were positive in all these cases.

TABLE 6.—ANALYSIS OF VARIANCE OF SURVIVORS OF ZYGOSACCHAROMYCES SPECIES HEATED AT GIVEN TEMPERATURES FOR VARYING LENGTHS OF TIME

Temperature	50° C. (122° F.)		60° C. (140° F.)		65° and 70° C. (149° and 158° F.)	
Variance due to	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Species	3	34.2489*	1	2.1079	3	10.4046
(1)Heating time	4	23.6919*	3	77.9822*	2	65.0959*
Species × (1)heating time	12	1.0184*	3	2.7682*	6	3.1614*
Error between tubes	79†	0.0145*	32	0.0383*	47†	0.3127*
Error within tubes	93‡	0.0029	40	0.0147	58††	0.0139

(1) "Heating time" becomes "temperature" for columns headed 65° and 70° C.
† 1 D.F. lost in filling missing reading.
‡ 7 D.F. lost in filling missing readings.
†† 2 D.F. lost in filling missing readings.
* Significant at 5% level.

From Table 6 it may be seen that there were no significant differences in the duplicate readings (within tubes), although the results obtained from the different tubes (between) showed significance.

Baker and McClung (2) have suggested that not less than 5 tubes should be tested at each time to obtain a reliable estimate of the effect of heating. The errors arising from heating 5 replicate tubes in the present experiment (between tubes) increase as the temperature increases. This indicates the necessity of testing a greater number of tubes at higher temperatures in order to obtain comparable reliability.

Species

When heated at 50° C. (122° F.) there were high significant differences between species, whereas the results obtained from heating at 60° C. (140° F.) and at 65° and 70° C. (149° and 158° F.) showed no significance. The results of testing at 60° C. (140° F.) are based on two species only, since the counts for M1 and 139 were negligible at the 5 min. period. The 40-min. heating results are omitted, as are also those obtained when momentary heating tests were carried out at 75° and 80° C. (167° and 176° F.).

The numbers of yeast cells used in the original inoculations were not the same, so that it is not possible to estimate the effect of heat on the species by comparing their means. Nevertheless, the interaction of species × heating time is highly significant showing that the species behaved in differential manner when heated at each temperature. The high significance when they were heated momentarily at different temperatures also bears out this fact. Table 7 elaborates the data used to bring out these points.

Heating Times and Temperatures

There were high significant differences when the tubes were heated at 50° C. (122° F.) and 60° C. (140° F.) for varying lengths of time and also when heated to 65° and 70° C. (149° and 158° F.) momentarily. These points are presented in detail in Table 7.

TABLE 7.—TOTAL LOG COUNT AND MEANS OF ZYGOSACCHAROMYCES SPECIES HEATED AT GIVEN TEMPERATURES FOR VARYING LENGTHS OF TIME

Temperature	Time	Species				Mean
		M1	J7	138	139	
50° C. (122° F.)	Check	50.7822	51.6365	64.2505	50.9712	5.4410*
	5 min.	47.5583	47.5444	59.2321	48.3544	5.0672
	15 min.	44.4407	45.6774	59.1183	36.3978	4.6408
	30 min.	37.9648	39.7242	53.6491	34.5905	4.1482
	60 min.	29.7575	35.5772	51.7670	25.2225	3.4831
60° C. (140° F.)	Check		51.9993	63.5735		5.7786†
	5 min.		35.1984	31.5446		3.3372
	10 min.		26.2078	23.8361		2.5022
	20 min.		6.9614	14.3985		1.0680
	Check	46.3530	52.6731	55.1064	48.4985	5.0659‡
65° C. (149° F.)	Moment.	32.1821	38.6774	43.3990	36.8957	3.7788
70° C. (158° F.)	Moment.	18.1329	13.8771	39.1379	29.4288	2.5144

* Necessary difference for significance of means (P = 0.05) 0.4917.
† Necessary difference for significance of means (P = 0.05) 1.6742.
‡ Necessary difference for significance of means (P = 0.05) 0.8662.

Times and Temperatures for "Commercial Sterility"

Experimental results have proven that absolute sterility is not practicable, since a few viable cells survive very severe heating. Therefore, the term used here is "commercial sterility", which is considered as embodying such time and temperature relationships as will leave the remaining honey with a count of less than 10 yeasts per ml.

To obtain definite information as to how long and to what temperatures it is necessary for inoculated honey to be heated to kill the majority of the organisms, the straight line formulae used in determining the curves in Figures 1 to 6 have been used to determine "commercial sterility". These times and temperatures are presented in Table 8.

TABLE 8.—TIMES AND TEMPERATURES REQUIRED TO OBTAIN "COMMERCIAL STERILITY" OF HONEY INOCULATED WITH ZYGOSACCHAROMYCES SPECIES

		Species			
		M1	J7	138	139
At 50° C. (122° F.)	Orig. count	121,000	153,300	2,672,000	126,200
	Time in min.	104.2	173.0	335.8	98.7
At 60° C. (140° F.)	Orig. count	126,900	159,400	2,288,500	115,600
	Time in min.	*	18.7	28.8	*
Momentary heating	Orig. count	43,800	186,100	324,900	73,000
	Temp.	74.7° C. (166.5° F.)	72.7° C. (162.9° F.)	81.7° C. (179.1° F.)	84.3° C. (183.7° F.)

* In the experimental work "commercial sterility" was obtained in 5 min.

It must be borne in mind that the data used in establishing the curves in Figures 7 to 10 are scanty, but they have been used in establishing the "flash" temperatures as indicated.

Since the time and temperature data in Table 8 have been calculated without taking into consideration the lag periods, or preheating times, as presented in Table 2, it is obvious that the times mentioned will vary in any system which has a different lag period.

II. By comparing the results in Table 4, it is obvious that the four species of Zygosaccharomyces used throughout this investigation were among those species most easily destroyed by heating to 75° C. (167° F.). Culture 40–60 was most resistant, but this yeast gave negative results when tested qualitatively.

The question arises as to whether yeast 40–60 is a distinct species. Fabian and McCullough (14) found that the gonidial form of *Zygosaccharomyces mandshuricus* displayed chromogenesis and produced acid fermentation with no gas. When tested for carbohydrate metabolism, culture 40–60 was found to produce acid in dextrose and sucrose, but none with maltose. No gas was produced with any of the sugars. On 15% honey agar slants growth was abundant, filiform, raised, glistening and smooth. This culture was isolated from a plate that had been inoculated with J7, and while dissociation has not been observed with this species, two distinct forms of 139 were encountered during the investigation. They are probably the smooth (S) and rough (R) forms illustrated in Plate I, 2 and 3. The "S" form was the one employed in all comparative tests.

Since other cultures (A2 and BK5) isolated from honey by the author show particular resistance to heat, it is not to be conclusively affirmed that data based on results obtained from species M1, J7, 138, and 139 are sufficiently comprehensive for "commercial sterility" under all circumstance.

SUMMARY AND CONCLUSIONS

Sterile honey heavily inoculated with fresh individual cultures of species of yeasts found by Lochhead and Farrell (21) to predominate in unfermented and fermented honey has been subjected to heat at different temperatures for varying lengths of time.

The analysis of over 1000 samples of Canadian honey by Lochhead (20) and by the author (unpublished data) has shown that very few honeys, either unfermented or fermented, give a yeast count of over 100,000 yeasts per gram. Consequently, inoculations were made to approximate maximum natural infection.

"Commercial sterility" is suggested as a term to be used in connection with honey pasteurization. It has been demonstrated that certain yeasts are capable of withstanding extreme heat, therefore, "commercial sterility" has been used as a basis for estimating the time-temperature relationships. Based on the experimental data it would require from 1 hour and 40 min. to 5 hours and 30 min. to reduce the count to less than 10 yeasts per ml. at 50° C. (122° F.) depending on the species and the initial inoculation. Different species acted differently in rate of dying so that this too, is a factor. At 60° C. (140° F.) the time necessary to obtain "commercial sterility" would be less than 30 min.

The temperature to be applied to honey to obtain "commercial sterility" by "flash pasteurization" methods is probably very near to 80° C. (176° F.) but will vary according to the length of time necessary to bring the honey to that temperature.

It is possible that particular heat resistant cells may give rise to strains of high resistance, therefore, any system in which honey is being heated for the destruction of yeasts should be cleaned thoroughly at frequent intervals, in order to avoid possible incorporation of such strains in honey which is being pasteurized.

ACKNOWLEDGMENTS

The author acknowledges the kindness of Dr. G. H. Duff, Department of Botany, University of Toronto, for supervision of the experimental work and the assistance of Dr. A. G. Lochhead, Division of Bacteriology and Dairy Research, Science Services, Department of Agriculture, Ottawa, in supplying cultures and in preparing the manuscript.

Mr. F. S. Nowosad, Forage Plants Division, Experimental Farms Service, Department of Agriculture, Ottawa, and Dr. J. W. Hopkins, Division of Biology and Agriculture, National Research Council, Ottawa gave valuable advice regarding the statistical treatment of results.

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THE ANALYSIS OF COVARIANCE BY THE METHOD OF INDIVIDUAL COMPARISONS[1]

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[Received for publication January 12, 1942]

Experiments of factorial design have many advantages, the chief one being that a maximum of information is obtained from a minimum of biological material. Fisher (3) and Yates (5) have outlined the basis and principles underlying this type of design. Brandt (1) has shown that as an experiment is made more complex, it becomes more efficient and the findings more widely applicable. However, as an experiment becomes more complex, the necessary calculations for a variance or covariance analysis become more laborious. The calculations may be reduced considerably by segregating an appropriate sum of squares for each degree of freedom. This method as applied to variance analysis has been discussed by many investigators and recently by Brandt (2) who illustrates with several examples. The purpose of this paper is to show that this method is also adaptable to covariance analysis.

The data used herein are taken from a fattening test previously reported upon (4) and the design is typical of those used at this institution for this type of work. In these tests, the variables considered were initial body weight, initial percentage fat, feed consumption, gain in body weight, and increase in percentage fat. For the purpose of this paper the latter two only will be used. The comparisons to be made are given in Table 1.

TABLE 1.—OUTLINE OF FATTENING TEST

Feed	Supplement	Temperature	Feed	Supplement	Temperature
A	*x*	40° F.	*B*	*x*	40° F.
A	*x*	50	*B*	*x*	50
A	*x*	60	*B*	*x*	60
A	*y*	40	*B*	*y*	40
A	*y*	50	*B*	*y*	50
A	*y*	60	*B*	*y*	60

If a treatment is considered as one feed with one supplement at one temperature then from the above table it will be seen that there are $2 \times 2 \times 3 = 12$ different treatments in the whole experiment. Each treatment is replicated 12 times since there are 12 birds in each of the smallest subclasses. The variance and covariance analyses are shown in Table 2.

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TABLE 2.—ANALYSIS OF VARIANCE AND COVARIANCE FOR GAIN IN BODY WEIGHT AND INCREASE IN PERCENTAGE FAT

Variance due to	D.F.	Gain in weight	Increase % fat	Covariance
Between feeds	1	106166.53	2.40	− 504.77
Between supplements	1	1720468.53	235.08	20111.13
Between temperatures	2	45343.53	70.83	1458.76
Interactions:				
F × S	1	7803.47	55.94	660.66
F × T	2	7969.47	116.75	925.76
S × T	2	25448.47	152.16	1918.91
F × S × T	2	147628.53	653.40	− 9757.17
Error	132	1871098.00	10064.25	28004.97
Total	143	3931926.53	11350.81	42818.25

These analyses are calculated in the usual manner. If there are many variables to be considered then the analyses become very laborious and time consuming. These calculations may be reduced considerably by segregating a sum of squares for each degree of freedom. This method of partitioning is shown in Table 3.

There are many sets of comparisons that might be used but those given in Table 3 will serve as an example. Since feed A is compared with feed B, all the groups receiving the former treatment are assigned a value of +1 and the latter a value of −1. The sum column for each row or comparison is the difference between the sums of the positive and negative values. Thus for the feed comparison, the sum is (5490 + 5086 + 5503 + 3276 + 2404 + 1999) − (5439 + 4233 + 3922 + 1531 + 2296 + 2427) = 3910. The divisor column is obtained by summing the squared coefficients $[(+1^2) + (+1^2) \ldots\ldots + (-1^2) + (-1^2) = 12]$, and since each group is replicated 12 times the sum of the squared coefficients of each row must be multiplied by 12. There are two conditions that must be fulfilled in setting up such a table. First, the sum of the positive and the negative values in each row must be zero, and second, the sum of the products of corresponding values in each combination of any two lines must be zero.

As noted above, all observations in this experiment are replicated and therefore there is a true error term ("within" variance). Under these conditions nothing is to be gained by evaluating the individual degrees of freedom for error and it is necessary therefore to determine the sums of squares of the deviations from the group mean for each of the 12 groups in the ordinary manner. As there are 12 birds in each group there are 11 degrees of freedom in each or a total of 132 in the 12 groups. An example of such a calculation is given under Table 3. The total sum of squares is the sum of the individual sums of squares calculated in Table 3 plus the error sum of squares. The covariance analysis is the product of the two appropriate sums i.e. for the between feed comparison $\frac{3910 \times -18.59}{12 \times 12} = \frac{-72686.90}{144} = -504.77$. The covariance for error is calculated in the usual way.

TABLE 3.—SEGREGATION OF THE APPROPRIATE SUM OF SQUARES AND CROSS PRODUCTS FOR INDIVIDUAL DEGREES OF FREEDOM

Group	$Ax40$	$Ax50$	$Ax60$	$Ay40$	$Ay50$	$Ay60$	$Bx40$	$Bx50$	$Bx60$	$By40$	$By50$	$By60$	Divisor	Gain in weight: Sum	Gain in weight: (Sum)²/Divisor	Increase in % fat: Sum	Increase in % fat: (Sum)²/Divisor	(Gain in weight)(Increase in % fat): Cross products	(Gain in weight)(Increase in % fat): Cross products/Divisor
Increase in % fat*	384.93	276.50	272.97	239.54	253.82	304.17	281.45	295.66	321.71	295.75	293.24	262.71							
Gain in body weight*	5490	5086	5503	3276	2404	1999	5439	4233	3922	1531	2296	2427							
Feed	+1	+1	+1	+1	+1	+1	−1	−1	−1	−1	−1	−1	12×12	3910	106167.36	− 18.59	2.40	−72686.90	−504.77
Supplement	+1	+1	+1	−1	−1	−1	+1	+1	+1	−1	−1	−1	12×12	15740	1720469.44	183.99	235.09	2896002.60	20111.13
Temperature																			
Linear effect T_1	−1		+1	−1		+1	−1		+1	−1		+1	8×12	−1885	37012.76	− 40.11	16.76	75607.35	787.58
Quadratic effect T_2	+1	−2	+1	+1	−2	+1	+1	−2	+1	+1	−2	+1	24×12	1549	8331.25	124.79	54.07	192463.25	671.18
															45344.01		70.83		1458.76
Interaction																			
$F \times S$	+1	+1	+1	−1	−1	−1	−1	−1	−1	+1	+1	+1	12×12	1060	7802.78	89.75	55.94	95135.00	660.66
$F \times T_1$	−1		+1	−1		+1	+1		−1	+1		−1	8×12	− 643	4306.76	− 54.55	31.00	35075.65	365.37
$F \times T_2$	+1	−2	+1	+1	−2	+1	−1	+2	−1	−1	+2	−1	24×12	1027	3662.25	157.15	85.75	161393.05	560.39
															7969.01		116.75		925.76
$S \times T_1$	−1		+1	+1		−1	−1		+1	+1		−1	8×12	− 1123	13136.76	−103.29	111.13	115994.67	1208.28
$S \times T_2$	+1	−2	+1	−1	+2	−1	+1	−2	+1	−1	+2	−1	24×12	1883	12311.42	108.69	41.02	205680.09	710.57
															25448.18		152.15		1918.85
F × $S \times T_1$	−1		+1	+1		−1	+1		−1	−1		+1	8×12	3703	142835.51	−249.89	650.47	−925342.67	−9638.99
F × $S \times T_2$	+1	−2	+1	−1	+2	−1	−1	+2	−1	+1	−2	+1	24×12	− 1175	4793.84	28.97	2.91	− 34039.75	− 118.19
															147629.35		653.38		−9757.18

* Sum for each of smallest subclasses.

Calculation of error sum of squares (Ax40)

$$\begin{aligned} \Sigma x &= 5490 \\ n &= 12 \\ \Sigma x^2 &= 2750136.00 \\ (\Sigma x)^2/n &= 2511675.00 \\ \Sigma(x - \bar{x})^2 &= 238461.00 \end{aligned}$$

ACKNOWLEDGMENT

The writer wishes to thank Dr. A. E. Brandt, Chief, Conservation Experiment Station Division, United States Department of Agriculture Washington, D.C. for assistance in the preparation of this manuscript.

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BOOK REVIEW

ECOLOGICAL CROP GEOGRAPHY. By Karl H. W. Klages. xviii + 615 pages, 108 maps and charts. Published by The Macmillan Company in Canada, 1942. ($4.50).

An attempt to bring together in a single volume a knowledge of the various principles which determine the distribution patterns of crop plants, this book is intended to fill a need long felt by agronomists, economists, geographers and other workers.

Professor Klages divides his book into four major sections, in each of which a different approach to the subject is made.

Part I, entitled "The Social Environment of Crop Plants" relates the economic history of agriculture from its earliest primitive beginnings to its modern commercial, mechanized, and highly specialized phases. The interrelation of crop and population distributions is also briefly dealt with, the author using freely many of the ideas of Bowman, Huntington, Taylor and other noted human geographers.

Part II, entitled "The Physiological Environment," is a rather general account of the interaction of the plant with the factors of its environment. Here Schimper's "ecological optimum" and Blackman's "limiting factors" provide the theoretical background for discussions of crop yield and variability, and plant adaptation.

Part III, "The Ecological Factors," is a more extended exposition of the principles of crop ecology. Here we find assembled a great deal of detailed information on the effects of moisture, temperature, light and wind upon the growth of crop plants. Included also, although rather reluctantly according to the author's own admission, is a chapter on the classification of climates, containing complete sets of continental maps of both Koppen's and Thornthwaite's climatic regions. In the reviewer's opinion this chapter adds greatly to the value of the book. The third section closes with an altogether too brief and sketchy chapter on soil factors.

Part IV, "The Geographical Distribution of Crop Plants" is much the longest section. A chapter is devoted to each of the principal groups of crop plants, such as the small grains, root crops, fibre crops, etc. Each crop in turn is discussed under various headings: commercial importance, historical, climatic relations, soil relations and distribution. Maps showing world distribution are given for all the important crops, many of them compiled from the latest available data, thus adding greatly to the value of the book.

It is to be expected in a compilation of this sort that the subject matter cannot be treated throughout with a uniform degree of detail. Neverthe-

less, there are some surprising omissions; for instance, the highly important topic of drainage is hardly mentioned while soil erosion is disposed of in less than three lines.

The book is to be commended as a pioneer in its field. The biblio graphical lists are excellent and carefully chosen and there are both subject and author index lists. This volume will furnish the crop production expert with a key to much geographical literature while to the geographer it will provide access to a great deal of technical agricultural information. It should attain wide use as text and reference book.

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